

**Case Nos. 17-70810, 17-70817**

**UNITED STATES COURT OF APPEALS  
FOR THE NINTH CIRCUIT**

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NATIONAL FAMILY FARM COALITION, et al.,  
*Petitioners,*

v.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY, et al.,  
*Respondents,*  
DOW AGROSCIENCES LLC,  
*Respondent-Intervenor.*

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NATURAL RESOURCES DEFENSE COUNCIL, INC.,  
*Petitioners,*

v.

SCOTT PRUITT, et al.,  
*Respondents,*  
DOW AGROSCIENCES LLC,  
*Respondent-Intervenor.*

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On Petition for Review from the  
United States Environmental Protection Agency

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**PETITIONERS' EXCERPTS OF RECORD VOLUME II**

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April 11, 2018

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<sup>2</sup> Respondent U.S. Environmental Protection Agency (EPA) did not produce, but only provided hyperlinks to, many publicly available documents. *See* Case No. 17-70810, ECF No. 56-3. For the Court's convenience, Petitioners have produced in the Excerpts of Record, in whole or relevant part, those hyperlinked documents cited in their briefs.

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<sup>4</sup> The documents bearing the number “6490-DVD” were cited in NRDC’s comments (No. 6490), and submitted to EPA via DVD on December 1, 2016.



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<sup>5</sup> This document was submitted to the docket for the previous Enlist Duo registration (EPA Docket No. EPA-HQ-OPP-2014-0195-3133), which is incorporated into the current Administrative Record. *See* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0195-3133> (last visited Apr. 10, 2018). However, the URL is omitted from EPA's Certified Amended Index, Case No. 17-70810, ECF No. 56-3.

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4/1/2007	445	USFWS. 2007. Indiana Bat ( <i>Myotis sodalis</i> ) Draft Recovery Plan: First Revision. U.S. Fish and Wildlife Service, Great Lakes-Big Rivers Region - Region 3, Fort Snelling, Minnesota. <a href="http://www.fws.gov/northeast/nyfo/es/IndianaBatapr07.pdf">http://www.fws.gov/northeast/nyfo/es/IndianaBatapr07.pdf</a> (continued in Volume X)	2224-2300

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1/23/2004	487, 6832	U.S. Environmental Protection Agency. 2004. Overview of the Ecological Risk Assessment Process in the Office of Pesticide Programs, U.S. Environmental Protection Agency. Listed and Threatened Species Effects Determinations.	2484-2575
12/1/1993	484, 6829, 6830	U.S. Environmental Protection Agency. 1993. Wildlife Exposure Factors Handbook EPA/600/R-93/187a, Office of Research and Development, Washington, DC (1 and 2) (continued in Volume XI)	2576-2590

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12/1/1993	484, 6829, 6830	U.S. Environmental Protection Agency. 1993. Wildlife Exposure Factors Handbook EPA/600/R-93/187a, Office of Research and Development, Washington, DC (1 and 2) (continued in Volume XII)	2591-2885


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12/1/1993	484, 6829, 6830	U.S. Environmental Protection Agency. 1993. Wildlife Exposure Factors Handbook EPA/600/R-93/187a, Office of Research and Development, Washington, DC (1 and 2)	2886-3147
1/15/1993	2206	Health Effects Division Chapter of the Reregistration Eligibility Decision for Glyphosate (EPA) [excerpt]	3148-3180





## Comment submitted by William Troxell, Executive Secretary, Pennsylvania Vegetable Growers Association

The is a Comment on the **Environmental Protection Agency (EPA)**  
Other: **Public Participation for Evaluation of 2,4-D Choline Salt  
Herbicide on Enlist Corn, Soybeans, and Cotton**

For related information, [Open Docket Folder](#) 

Comment Period Closed  
Dec 1 2016, at 11:59 PM ET

**ID:** EPA-HQ-OPP-2016-0594-0582

**Tracking Number:** 1k0-8tbx-jk1j

### Document Information

**Date Posted:**

Dec 14, 2016

**RIN:**

Not Assigned

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### Comment

The Pennsylvania Vegetable Growers Association continues to be opposed to the registration of any of the products containing formulations of dicamba and/or 2,4,D for use on genetically engineered crops. This family of chemistry has had a long history of volatilization problems despite a number of reformulations over the years. Our concern is that farmers will opt for the older, cheaper products to use on their genetically-modified soybeans. These products can volatilize and impact crops hundreds of feet from the application sites. In addition, we will see an increase in these products used on corn acres as well if it will no longer damage soybeans.


By labeling these products, it will put vegetable and small fruit farmers in the difficult position of seeking damages from a neighbor. This will unnecessarily strain neighbor relations. This is an especially important issue in Pennsylvania because our vegetable and small fruit farms are interspersed with field crop farms across the state. This is even more of an issue in our prime vegetable growing areas like Lancaster County which already has issues with 2,4,D damage from lawn and turf applications.

As an Association, we are not opposed categorically to genetically modified crops and other biotechnology advances in agriculture. We do, however, feel that this new use of 2,4,D has the potential to cause neighboring crop damage situations unnecessarily. We feel Enlist should not be labelled for use in Pennsylvania.



## Comment submitted by John E. Peck, Executive Director, Family Farm Defenders

The is a Comment on the **Environmental Protection Agency (EPA)**  
Other: **Public Participation for Evaluation of 2,4-D Choline Salt  
Herbicide on Enlist Corn, Soybeans, and Cotton**

For related information, [Open Docket Folder](#) 

Comment Period Closed  
Dec 1 2016, at 11:59 PM ET

**ID:** EPA-HQ-OPP-2016-0594-0518

**Tracking Number:** 1k0-8tbs-o2c2

### Document Information

**Date Posted:**

Dec 14, 2016

**RIN:**

Not Assigned

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### Comment

Family Farm Defenders, which represents thousands of farmers across the U.S. including many who plant corn, soy, and cotton, is strongly opposed to this approval of 2,4-D Choline Salt Herbicide for use on Enlist crops. We have not seen any great yield gain as promised with in patented crop varieties that are engineered to withstand pesticide treatments, and the expense of the seeds along with the required chemicals actually means many farmers end up losing money in the process. As well, the detrimental impacts on human health and the broader environment in the case of 2,4-D are well documented and this approval would represent a serious step backward in terms of improving the quality of life of family farmers, other rural residents, as well as the integrity of our soil, water, and wildlife resources. Some of members who are veterans of the Vietnam War are still recovering from their toxic exposure to 2,4-D (aka Agent Orange) in that conflict, and it does not make sense to expose our civilian population to the same threat when there a much better (and cheaper) ways available to control weed pressure in corn, soy, and cotton. - John E. Peck, executive director, Family Farm Defenders



## Comment submitted by Lisa Griffith, National Family Farm Coalition

The is a Comment on the **Environmental Protection Agency (EPA)**  
Other: **Public Participation for Evaluation of 2,4-D Choline Salt  
Herbicide on Enlist Corn, Soybeans, and Cotton**

For related information, [Open Docket Folder](#) 

Comment Period Closed  
Dec 1 2016, at 11:59 PM ET

**ID:** EPA-HQ-OPP-2016-0594-0647

**Tracking Number:** 1k0-8tcp-kt0k

### Document Information

**Date Posted:**

Dec 14, 2016

**RIN:**

Not Assigned

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### Comment

As a coalition representing thousands of family farmers and ranchers, community-based fishermen, rural residents and fair food advocates, we oppose any and all expansion of Enlist Duo crops. The wider use of the lethal combination of glyphosate and 2,4-D to kill weeds associated with genetically engineered corn, soy and cotton is a frightening proposition to all of us.

We know of more and more pesticide drift resulting in the loss of non-GE crops to the tune of tens and hundreds of thousands of dollars and even murder (in a recent case of dicamba drift, per <http://www.agriculture.com/news/crops/off-target-dicamba-movement-prompts-arkansas-shooting>). These chemical cocktails also lead to more superweeds that resist these pesticides and are the very possible causes of more health problems, including cancers, fertility issues, the irritation and inflammation of eyes and skin, hives, nausea, vomiting, throat irritation, headache, dizziness, coughing and difficulty breathing (see <https://www.panna.org/sites/default/files/24D-factsheet.pdf>).


We hope you will take our concerns to heart as you consider allowing more herbicide-resistant crops to be planted despite research suggesting that they are ultimately a step backward (<http://rodaleinstitute.org/assets/FSTbooklet.pdf>).

Thank you for the opportunity to offer comments during this month-long period.



## Comment submitted by B. Day

The is a Comment on the **Environmental Protection Agency (EPA)**  
Other: **Public Participation for Evaluation of 2,4-D Choline Salt**  
**Herbicide on Enlist Corn, Soybeans, and Cotton**

For related information, [Open Docket Folder](#) 

Comment Period Closed

Dec 1 2016, at 11:59 PM ET

**ID:** EPA-HQ-OPP-2016-0594-0069

**Tracking Number:** 1k0-8tan-cm15

### Document Information

**Date Posted:**

Dec 6, 2016

**RIN:**

Not Assigned

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### Comment

I am Bill Day, vineyard owner of Buena Suerte Vineyards LLC, located west of Meadow, TX, in Terry County. My vineyard is 6 years old. In every year of its existence, I have found grape vine leaves showing evidence of herbicide contamination, specifically from 24D, paraquat and Roundup. My 50 acres is in the center of my 640 acre farm. My closest vines are 1/4 mile from my closest neighbor. Since 24D is particularly known for volatilizing and drifting for miles, I am worried about this latest formulation of a known killer of grape vines. Since the rest of my farm is organic, none of the damage is from my own usage. The damage is coming from outside my 640 acres. Thus far, it has only stunted growth of my vines. My vineyard consultant, Bobby Cox has brought samples of damaged leaves to my attention as has Cliff Bingham, my farmer and grape grower. We have all witnessed the damage. Bobby and Cliff have found damage in their own vineyards. Due to the drift issue of 24D in every form of its existence, it is a danger to my vineyard and also my other organic crops. When will the USDA take action to protect my crops? TDA is NOT protecting my vineyard. Stop the spread of 24D, paraquat and Roundup.



Docket No. EPA-HQ-OPP-2016-0594

Environmental Protection Agency Docket Center  
1200 Pennsylvania Ave., NW  
Washington, D.C. 20460

SUBMITTED VIA REGULATIONS.GOV

December 1, 2016

**Comments from Center for Food Safety on the following EPA proposed decisions, Docket ID No. EPA-HQ-OPP-2016-0594:**

- 1) New decision on use of Enlist Duo on herbicide-resistant corn and soybeans in 15 states following Court remand**
- 2) New decision to expand use of Enlist Duo on herbicide-resistant corn and soybeans in an additional 19 states**
- 3) Decision on new use of Enlist Duo herbicide-resistant cotton in the 34 states corresponding to those proposed for corn and soybeans**

### **Science Comments I: Environmental Impacts**

By: Martha L. Crouch, Ph.D., Science Consultant

Center for Food Safety (CFS) appreciates the opportunity to comment on EPA's proposed registration decisions regarding the use of Dow AgroScience's (DAS) Enlist Duo formulation GF2726 herbicide containing active ingredients 2,4-dichlorophenoxyacetic acid (2,4-D) choline salt and glyphosate dimethylammonium salt (glyphosate) on DAS's Enlist corn, soybeans, and cotton genetically engineered (GE) to be resistant to 2,4-D, glyphosate, and other herbicides.<sup>1</sup>

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<sup>1</sup> "Dow AgroSciences LLC, the manufacturer and registrant of 2,4-D choline salt, submitted an application to the U.S. Environmental Protection Agency (EPA) to register Enlist Duo formulation GF2726 for application to 1) Enlist corn and field corn (containing DAS 68416-4 trait), 2) Enlist soybean (containing DAS 68416-4), and 3) Enlist cotton (containing DAS 81910-7). All of these crops contain genetic traits that make them tolerant to the herbicides 2,4-D as well as glyphosate (both herbicides are active ingredient components of GF2726). The genetic traits on the resistant crops allow for application of 2,4-D choline (in this case GF2726) to herbicide-tolerant cotton, corn and soybeans later in the growing season (later growth stages) than conventional varieties of these crops." (EPA-HQ-OPP-216-0594- 0013 at 4). In addition to 2,4-D resistance, Enlist soybean and cotton are also genetically engineered

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CFS is submitting 3 new comments to Docket ID No. EPA-HQ-OPP-2016-0594: legal comments and two science comments, with associated documents. Studies are submitted as pdf files with “name and date” as cited in the comments. Previous comments to EPA and USDA on 2,4-D use with HR crops are submitted as Exhibits.

As described by EPA, “On October 15, 2014, EPA registered Enlist Duo—an herbicide formulation containing the dimethylammonium salt of glyphosate (glyphosate) and the choline salt of 2,4-dichlorophenoxyacetic acid (2,4-D)—for use on Enlist corn and soybeans, which has been genetically engineered (GE) for resistance to glyphosate and 2,4-D, in six states: Illinois, Indiana, Iowa, Ohio, South Dakota, and Wisconsin. On March 31, 2015, EPA amended that registration to allow use of Enlist Duo on GE corn and soybean in the nine additional states of Arkansas, Kansas, Louisiana, Minnesota, Mississippi, Missouri, Nebraska, North Dakota, and Oklahoma. Due to missing data regarding the synergy between 2,4-D and glyphosate, a court ordered a remand of EPA’s proposed registration of Enlist Duo for use on GE corn and soybean in all fifteen states. EPA now seeks to amend the registration to register Enlist Duo on GE cotton, which has also been genetically engineered for resistance to glyphosate and 2,4-D, in all the states where use on GE corn and soybean have been registered, plus the additional states of Alabama, Arizona, Colorado, Delaware, Florida, Georgia, Kentucky, Maryland, Michigan, North Carolina, New Jersey, New Mexico, New York, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, and West Virginia. In addition, EPA seeks to include the expansion of the use of Enlist Duo on GE corn and soybeans to include the nineteen additional states” ((EPA-HQ-OPP-2016-0594-0015 at 1). These comments are in response to: 1) EPA’s new decision for use of Enlist Duo on GE herbicide-resistant corn and soybeans in the original fifteen states following the court’s remand, 2) EPA’s new decision to expand use of Enlist Duo on GE herbicide-resistant corn and soybeans in an additional nineteen states, and 3) EPA’s decision on new use of Enlist Duo on GE herbicide-resistant cotton in the thirty-four states corresponding to those proposed for corn and soybeans.

**I. EPA’s New Use Approvals Will Increase the Amount of 2,4-D Used in the U.S., Which Will Cause Unreasonable Adverse Effects on the Environment.**

As in the previous registration for Enlist Duo herbicide applied on Enlist corn and soybeans in 15 states (Ex. C at 4-5), when assessing risks EPA ignores the large increase in overall use of 2,4-D that will result. With the proposed registration, much more 2,4-D will be

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to withstand the herbicide glufosinate, and Enlist corn is genetically engineered to withstand ACCase-inhibitor herbicides. Note that the glyphosate resistance trait in these crops was added by crossing it from other lines (e.g. Roundup Ready 2 Yield soybean). Also, DAS has another 2,4-D resistant soybean (DAS 44406-6) where traits for resistance to 2,4-D, glyphosate, and glufosinate were engineered together into one event; and this will be released in Enlist E3 soybean varieties in the near future, according to DAS (DowAgroSciences 2016).

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used in US agriculture because Enlist Duo will be used on Enlist cotton<sup>2</sup> in addition to corn and soybeans, and in 34 states. These 34 states encompass most of the US, including the massive Mississippi River and Great Lakes watersheds, already severely degraded by agricultural chemicals and practices (e.g. Fausti et al. 2015). The large increase in 2,4-D and total herbicide use resulting from this registration action will add to the unacceptable pesticide load already in US soil, water and air, and in organisms (Beketov et al. 2013, de Oliveira et al. 2016, Lundgren and Fausti 2015, Stehle and Schulz 2015), with impacts that must be realistically assessed by EPA.

In addition to the large increase in total land area that will be treated with 2,4-D if this proposed registration is approved, the new use of 2,4-D on Enlist cotton will increase 2,4-D use within local and regional watersheds where cotton is grown alongside or in rotation with Enlist corn and soybeans. The impacts of having more adjacent acres of land simultaneously or sequentially treated with 2,4-D in these smaller regions must also be assessed (Ex. B-2 at 59).

EPA makes the unrealistic assumption that, with mandated mitigations in place, non-target organisms will not be exposed to 2,4-D from Enlist Duo at levels that exceed “Agency risk concern levels for listed and non-listed species of all taxonomic groups” unless those organisms are actually within the boundaries of the field being sprayed (EPA-HQ-OPP-2016- 0594-0013 Ecological Risk Assessment at 6). We have commented previously about why EPA’s over-reliance on mitigation measures to protect non-target species is scientifically unsound (Ex. B-2 at 60 – 61, Ex. A-3 at 3 – 5), and our previous critiques are valid for the current registration as well.

## **II. EPA Did Not Adequately Analyze the Synergy Between Enlist Duo and Other Pesticide Formulations and Adjuvants That Will Be Used in Conjunction with Enlist Duo.**

Based on scientific studies showing that some mixtures are significantly more toxic to particular organisms than the active ingredient alone, CFS has consistently argued that toxicity of end-use pesticide formulations must be determined and taken into account when assessing risks (Ex. B-2 at 5, 14 – 17, 41 - 44; see also new studies such as: amphibians, Risolli et al. 2016, Wagner et al. 2016; insects, Ciarlo et al. 2012, Freydier and Lundgren 2016, Mullin et al. 2015, 2016; grapes, Mosheni-Moghadam et al. 2016). This is especially important for determining

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<sup>2</sup> “Cotton with 700,000 lb ai of 2,4-D applied to 10 to 15 percent of cotton acres represents only a modest use of 2,4-D” (APHIS EA at 91). “Dow estimates that the increase may reach between 6.2 and 9.3 million pounds, assuming 0.875 lb per acre applied” (APHIS EA at 102). “Under the Preferred Alternative in this EA, an increase in 2,4-D use on cotton is also expected. Use is predicted to occur on 15-45% of cotton acres within ten years, using Dow’s estimate of a 5.7-fold increase in 2,4-D, based mainly on market share and potential for cross-licensing of the trait (see Scenario 2, Appendix 3, of this EA).” (USDA APHIS 2015 at 94, Appendix 3. Dow’s Estimate of Future Enlist Duo Use and Planting of DAS-81910-7 Cotton).



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whether mitigations such as in-field buffers during applications are protective of non-target organisms.

As described by EPA (EPA-HQ-OPP-2016-0594-0015 Registration at 1 - 3), after Enlist Duo was initially registered, and while the registration was being challenged in court, EPA learned that DAS had made claims of synergy between 2,4-D choline and glyphosate in patents, and that this might make Enlist Duo more toxic to plants than EPA had predicted. DAS had not disclosed synergy studies to EPA during the registration process. The court granted EPA a remand of the registration in order to determine the implications of the synergy claim for its risk assessments. EPA apparently examined existing DAS synergy data, but in the end required DAS to perform new, straightforward plant toxicity tests rather than more complicated synergy tests with the Enlist Duo formulation (EPA-HQ-OPP-2016-0594-0007 Plant Toxicity). Results showed no increased toxicity of Enlist Duo relative to 2,4-D amine alone, the surrogate active ingredient used in EPA's risk assessments for plants, so EPA is now proposing to reinstate the original registration without significant changes to the risk determinations or mitigation measures, and to expand the registration to new states and a new crop, Enlist cotton.

The decision by EPA to do toxicity tests on plants with the Enlist Duo formulation rather than a surrogate active ingredient alone is not adequate for determining risks from interactions between pesticide components in the proposed registration. There are other ingredients added by the end user just before application – other pesticide active ingredients (Donley 2016), and various adjuvants that change qualities of pesticide solutions (e.g. Curran and Lingenfelter 2009) – that also may change the toxicity of 2,4-D choline to plants and also to other taxa (Tornisiello et al. 2013). For example, in a recent study of herbicide toxicity to a beneficial lady beetle, a particular 2,4-D formulation was almost as toxic as the insecticide added as a positive control, and the toxicity was due mostly to an undisclosed “inert” ingredient (Freydier and Lundgren 2016).

Some of the other ingredients that are likely to be added to Enlist Duo when used on Enlist corn, soybeans and cotton have been claimed in patents or shown in studies to increase toxicity of 2,4-D, and thus must be tested to see if their use changes EPA's risk assessments before being allowed to be used in tank mixes or formulations. Examples include the herbicide glufosinate (Merchant et al. 2013, 2014; Mann et al. 2014), which is being marketed by DAS for use with Enlist soybeans and cotton (Dow AgroSciences 2016)<sup>3</sup>. Also, some adjuvants that

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<sup>3</sup> “Glufosinate applied together with 2,4-D may be more efficacious than either herbicide singly when controlling taller Palmer amaranth (Merchant et al., 2014b), so in situations where this applies, glufosinate use may increase when DAS-81910-7 cotton has nonregulated status. APHIS concludes that 2,4-D-and glufosinate-resistant varieties would at least replace some of these glufosinate-resistant-only varieties, and, if both herbicides were needed together for control of certain GR weeds, it may result in some additional glufosinate applications.” (USDA APHIS 2015 at 102).



contain organosilicones (Sussman n.d.), have been shown to be toxic to bees (Ciarlo et al. 2012, Mullin et al. 2015, 2016). Adjuvants containing organosilicones are already on the “approved” list of products to tank-mix with Enlist Duo (e.g. Event, FS Certin, R-11, Syntact, and Zenith: Dow Chemical 2016, Coastal AgroBusiness 2016). Some approved adjuvants are also accompanied by claims of increased herbicide “activity” (i.e. toxicity to plants) (e.g. Precision Laboratories 2016). Some agricultural analysts are predicting that adjuvant use will be common with Enlist crop systems and may spur development and use of new adjuvant products (Crummet 2016). Dow AgroSciences has a patent for new adjuvants and “inerts” that claims synergy with many pesticide active ingredients, including glyphosate and glufosinate (Babcock et al. 2013). EPA’s proposed registration only requires that tank-mix products undergo testing for whether they change the drift potential of Enlist Duo, not for whether they change toxicity (EPA-HQ-OPP-2016-0594-0015 Registration at 31).

### **III. EPA Did Not Adequately Analyze the Risks that Enlist Duo Poses to Pollinators and Other Organisms Beneficial to Agriculture.**

Risks to pollinators and other organisms beneficial to agriculture are inadequately assessed by EPA in the proposed registration. In addition to the deficiencies CFS has commented on before for use of Enlist Duo on Enlist corn and soybeans (pollinators: Ex. B-3 at 47 – 58, other beneficial organisms: Ex. B-3 at 45 – 47; Ex. A-3 at 5; see also new studies that show impacts on beneficial insects from over-control of weeds in agricultural fields: de Menezes and Soares 2016, DiTommaso et al. 2016), and the threats to pollinators from formulations (above), the proposed new use of Enlist Duo on cotton raises additional issues (Ex. D-2 at 70 – 73). EPA acknowledges that there are data gaps regarding risks to pollinators and that because Enlist Duo can be used on cotton during flowering, the pathway exists for impacts (EPA-HQ-OPP- 2016-0594-0013 at 9, 11 - 12, 59 – 60), but EPA falls short of requiring more data before registration. Nor does EPA consider the other ways beneficial organisms can be impacted by Enlist Duo use on Enlist cotton.

Cotton plants attract pollinators and other nectar- and pollen-eating animals, including honeybees and wild bees of several genera, beneficial wasps that parasitize herbivorous insects, and both beneficial and pest species of moths and butterflies (Röse et al. 2006). Not only are these insects attracted by the large flowers with both pollen and nectar, but also cotton plants have nectaries outside of the flowers that produce nectar both before and after flowers open, extending the season that cotton plants are attractive. Beneficial insects are thus likely to be exposed to residues and metabolites of 2,4-D, glyphosate, and other ingredients in Enlist Duo formulations and tank mixes directly, via contact with the spray and from residues in food, during a longer period than just when flowers are present. Other pesticides used on cotton already have contaminated cotton resulting in a pesticide cocktail for bees using the crop (Frazier

et al. 2015). Thus residues of all components of formulations and tank mixes need to be determined after Enlist Duo applications to Enlist cotton, and risks assessed.

**IV. EPA Did Not Adequately Analyze Impacts of Enlist Duo to Threatened and Endangered Species Listed Under the Endangered Species Act Due in Part to Its Erroneous Assumptions Regarding Off-site Movement of Enlist Duo.**

Expanding the number of states and including Enlist cotton in the Enlist Duo registration increases the number of listed species that must be considered in the Endangered Species Act (ESA) assessments. In ESA assessments in the proposed registration, EPA continues to make indefensible assumptions about exposure of threatened and endangered species and their critical habitats to Enlist Duo. EPA claims that only those ESA-listed species that directly inhabit corn and soybean fields have the potential to be affected by Enlist Duo used on Enlist crops, based on complete effectiveness of mitigation measures for off-site herbicide movement (EPA-HQ-OPP-2016-0594 at 72 – 76, Ex. B-2 at 60 – 61, Ex. A-3 at 3 - 5). For the listed species that EPA determines do inhabit corn and soybean fields (EPA-HQ-OPP-2016-0594 at 76 – 77, EPA-HQ-OPP-2016-0594-0014 at 2) EPA makes arbitrary and capricious assumptions about their biology that are likely to underestimate risks (Ex. A-3 at 2, 6 – 7, where CFS points out deficiencies in EPA’s assumptions with the examples of whooping cranes, Mississippi Sandhill cranes, American burying beetles, Indiana bats, Ozark bats, and Louisiana black bears.)

Additional examples of EPA’s similarly arbitrary and capricious assumptions about the biology of listed species inhabiting corn, soybean or cotton fields in the proposed registration include reptiles and amphibians, such as the Eastern massasauga rattlesnake (EPA-HQ-OPP-2016- 0594-0014 at 6), indigo snake, gopher tortoise, and Houston toad (EPA-HQ-OPP-2016-0594- 0013 at 97 – 100). EPA uses birds as surrogate species for reptiles and terrestrial phase amphibians, in spite of large differences in their biological characteristics ((Ex. B-2 at 58 - 59). For example, reptiles and amphibians are much more likely than birds to absorb pesticides through direct contact (Weir et al. 2014, Van Meter et al. 2014, 2015, 2016), with dermal intake of 2,4-D and glyphosate formulations shown to change biochemical markers of health in a toad (Lajmanovich et al. 2015). EPA only considers consumption of 2,4-D-contaminated food in the risk assessments, underestimating risk significantly.

For insects, EPA determined that for 2,4-D there will be “no direct toxic effects to terrestrial insects, even within the treated fields” based on acute toxicity studies with honeybees as a surrogate species. However, studies with some 2,4-D formulations have shown toxicity similar to insecticides on another beetle (Freydier and Lundgren 2016). Therefore, calculations of risks to the American burying beetle that do not take into account direct toxic effects of whole formulations (EPA-HQ-OPP-2016-0594-0013 at 100 – 101) may greatly underestimate actual risks.

As CFS has explained in previous comments, irrespective of the quality of assumptions made and data used by EPA in determining risks to listed species, EPA's use of FIFRA-based Risk Quotients and Levels of Concern to make ESA-based Effects Determinations is inappropriate because of differences between the laws and regulation of the Acts (CFS Legal Comments to this docket; Ex. A1, Ex. A-4). EPA is not the expert agency on the biology of listed species, so it must consult with the U.S. Fish and Wildlife Service and the U.S. Marine Fisheries Service if there is any effect at all, rather than attempt to make effects determinations itself.

## **V. Conclusion.**

In these comments, as well as previous comments, CFS has presented numerous science-based arguments demonstrating that EPA should reject the proposed registration of Enlist Duo for use on Enlist corn, soybeans, and cotton in all 34 states to protect the environment, pollinators, and threatened and endangered species. At a minimum, EPA should postpone its decision until it can conduct a comprehensive and critical scientific review of the impacts of using Enlist Duo on Enlist crops to avoid the significant potential harms CFS has identified in these and past comments.

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## WEED SCIENCE

### Weed Response to 2,4-D, 2,4-DB, and Dicamba Applied Alone or with Glufosinate

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#### ABSTRACT

Cotton tolerant of 2,4-D, glufosinate, and glyphosate or dicamba, glufosinate, and glyphosate is in development. This technology will give growers additional tools to manage glyphosate-resistant weeds. A field experiment was conducted across six environments in Georgia, North Carolina, and Tennessee to determine the response of 13- to 20-cm weeds to 2,4-D, 2,4-DB, and dicamba applied alone or mixed with glufosinate. Palmer amaranth (*Amaranthus palmeri* S. Wats) was controlled 59 to 78%, 68 to 80%, and 59 to 83% by 2,4-DB dimethylamine (560-1120 g ae ha<sup>-1</sup>), 2,4-D dimethylamine (530-1060 g a.e. ha<sup>-1</sup>), and dicamba diglycolamine (280-1120 g ae ha<sup>-1</sup>), respectively, and 74% by glufosinate ammonium (430 g ae ha<sup>-1</sup>). Control was improved (89-97%) with all auxin/glufosinate mixtures when compared to respective herbicides alone. Glufosinate controlled Benghal dayflower (*Commelina benghalensis* L.) only 68%; 2,4-D at 530 g ha<sup>-1</sup> and dicamba at 1120 g ha<sup>-1</sup> controlled this weed at least 90%. Combinations of glufosinate and auxin herbicides were beneficial when control by auxin herbicides was 90% or less. Carpetweed (*Mollugo verticillata* L.) control by auxin herbicides ranged from 50 to 66%; glufosinate alone or in mixtures completely controlled carpetweed. All treatments completely controlled morningglory (*Ipomoea* spp.). Auxin herbicides had no activity on grasses. Texas millet (*Panicum texanum* [Buckl.] R. Webster) and broadleaf signalgrass (*Brachiaria platyphylla* [Nash] R. Webster)

were controlled 89 to 90% by glufosinate alone. Both 2,4-D and 2,4-DB mixed with glufosinate reduced Texas millet control, and 2,4-D reduced broadleaf signalgrass control.

Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri* S. Wats) has drastically changed agronomic crop production throughout the southeastern U.S., most notably cotton production (Sosnoskie and Culpepper, 2012; Webster and Sosnoskie, 2010). Ninety-two percent of Georgia cotton growers hand-weeded 54% of their crop, spending an average of \$63.50 per hand-weeded hectare during 2010 (Sosnoskie and Culpepper, 2012). Hand-weeding is a secondary line of defense against this pest as these same growers apply more than \$150 ha<sup>-1</sup> in herbicides with applications beginning at burndown and continuing through cotton canopy closure. Additionally, these growers have reduced conservation tillage by 7%, increased cultivation to 43% of the hectares, and increased both the use of moldboard plows (100,000 ha during 2009 and 2010) and the use of secondary preplant tillage implements to incorporate herbicides (100,000 ha during 2010) in conventionally tilled systems (Culpepper et al., 2010; Price et al., 2011; Sosnoskie and Culpepper, 2012).

Effective control of Palmer amaranth in cotton has been achieved with glufosinate-based systems (Culpepper et al., 2009; Everman et al., 2007; Gardner et al., 2006; Whitaker et al., 2011). Glufosinate must be applied to small Palmer amaranth for consistently effective control (Coetzer et al., 2002; Culpepper et al., 2010). Palmer amaranth grows rapidly (Horak and Loughin, 2000) and growers are often unable to make timely applications. Another herbicide mixed with glufosinate might improve control of larger weeds. Herbicides that could potentially be mixed with glufosinate applied postemergence include MSMA, pyriithiobac, trifloxysulfuron, and fluometuron. MSMA has poor activity on Palmer amaranth, especially at rates that can be applied overtop of cotton (Culpepper, 2012). Moreover, combinations of MSMA plus glufosinate might be antagonistic (Koger et al., 2007). The ALS-inhibiting herbicides

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pyrithiobac and trifloxysulfuron can control Palmer amaranth (Branson et al., 2005; Culpepper and York, 1997). However, Palmer amaranth biotypes resistant to ALS-inhibiting herbicides are widespread across the Mid-South and Southeast (Heap, 2012; Wise et al., 2009). Fluometuron mixed with glufosinate has improved control of larger Palmer amaranth (Barnett et al., 2011), but postemergence (topical) application of fluometuron has been discouraged because it injures cotton, delays maturity, and sometimes reduces yield (Byrd and York, 1987; Snipes and Byrd, 1994).

Transgenic cotton resistant to 2,4-D is being developed (Braxton et al., 2010). The traits for resistance to these auxin herbicides will be stacked with traits conferring resistance to both glufosinate and glyphosate. Auxin herbicides are effective on a number of broadleaf weeds commonly infesting cotton (Green and Owen, 2011; Mueller et al., 2005), and they will be recommended in combination with glufosinate. It is important that Extension personnel and other advisors better understand the response of weeds to these herbicide mixtures.

## MATERIALS AND METHODS

The experiment was conducted in 2009 at Macon, Tift, and Colquitt counties in Georgia. Three experiments, separated by planting date, were conducted at the Macon County location. The experiment also was conducted in 2010 at Worth County, GA, Shelby County, TN, and Edgecombe County, NC. All sites

were fallow fields with naturally occurring weed populations being evaluated (Table 1). Plot sizes were 1.8 by 7.6 m in Georgia, 1.5 by 6.1 m in Tennessee, and 3 by 6.1 m in North Carolina. Soils in Georgia and North Carolina were loamy sands or sandy loams, whereas the soil in Tennessee a silt loam. The experimental design was a randomized complete block with four replications of each treatment. Treatments consisted of a factorial arrangement of two rates of glufosinate by 10 auxin herbicide and rate combinations. Glufosinate ammonium salt (Ignite 280 SL Herbicide, Bayer CropScience LP, Research Triangle Park, NC) was applied at 0 and 430 g ae ha<sup>-1</sup>. The auxin herbicide and rate combinations included the following: no auxin herbicide; the dimethylamine salt of 2,4-DB (Agri Star<sup>®</sup> Butyrac<sup>®</sup> 200 Broadleaf Herbicide, Albaugh, Inc., Ankeny, IA) at 560, 840, and 1120 g ae ha<sup>-1</sup>; the dimethylamine salt of 2,4-D (Nufarm Weedar<sup>®</sup> 64, Nufarm, Inc., Burr Ridge, IL) at 530, 800, and 1060 g ae ha<sup>-1</sup>; and the diglycolamine salt of dicamba (Clarity<sup>®</sup> Herbicide, BASF Corp., Research Triangle Park, NC) at 280, 560, and 1120 g ae ha<sup>-1</sup>. Herbicides were applied to weeds at the sizes indicated in Table 1 using CO<sub>2</sub>-pressurized backpack sprayers calibrated to deliver 140 L/ha at 165 kPa in Georgia and North Carolina or 140 L/ha at 207 kPa in Tennessee. Nozzles included DG11002 TeeJet<sup>®</sup> Drift Guard Flat Fan Spray Tips (TeeJet Technologies, Wheaton, IL) with 45.7-cm nozzle spacing in Georgia and North Carolina and TP8002 TeeJet<sup>®</sup> Flat Spray Tips (TeeJet Technologies, Wheaton, IL) with 50.8-cm nozzle spacing in Tennessee.

**Table1. Weed size<sup>z</sup> and density at time of herbicide application**

Location	Year	Weeds Present	Weed size at application cm	Weed density at application plants m <sup>-2</sup>
Macon County, GA <sup>y</sup>	2009	Palmer amaranth	15-20	125
Tift County, GA	2009	Palmer amaranth	18-25	7
		Carpetweed	13-18	4
Colquitt County, GA	2009	Pitted morningglory	18-23	4
		Benghal dayflower	15-20	22
		Broadleaf signalgrass	20	12
Shelby County, TN	2010	Palmer amaranth	20	65
Worth County, GA	2010	Texas millet	15-20	18
		Entireleaf morningglory	15-20	10
Edgecombe County, NC	2010	Broadleaf signalgrass	15 cm	60

<sup>z</sup> Size refers to height of Palmer amaranth, Benghal dayflower, broadleaf signalgrass, and Texas millet, diameter of carpetweed, and runner length of pitted morningglory and entireleaf morningglory.

<sup>y</sup> Three trials were conducted at the Macon site, each with Palmer amaranth at similar densities.

Weed control was estimated visually at 10, 20, and 30 d after herbicide application using a scale of 0 to 100, where 0 = no control and 100 = complete control (Frans et al., 1986). With weed responses being consistent across evaluation dates, only the 20-d evaluation is reported. Data were transformed to improve normality and homogeneity of variance and then analyzed using PROC Mixed of SAS (version 9.1; SAS Institute, Inc., Cary, NC). Site and replication were considered random effects, whereas treatments were considered fixed effects. When significant differences were noted, interaction means were present and post-hoc pair-wise comparisons were made using Tukey's HSD at  $P \leq 0.05$  to specifically compare auxin plus glufosinate mixtures to the respective auxin applied alone. Nontransformed comparisons are reported.

## RESULTS AND DISCUSSION

**Palmer Amaranth.** Glufosinate applied alone controlled Palmer amaranth only 74% (Table 2), a level of control expected when treating 20-cm tall plants (Coetzer et al., 2002). 2,4-D controlled Palmer amaranth 68, 79, and 80% when applied at 530, 800, and 1060 g ha<sup>-1</sup> respectively. Control by 2,4-DB at 840 and 1120 g ha<sup>-1</sup> and dicamba at 560 and

1120 g ha<sup>-1</sup> was similar to control by 2,4-D at 800 and 1060 g ha<sup>-1</sup>. At the lowest application rate of 530 g ha<sup>-1</sup>, 2,4-D was 9% more effective than 2,4-DB at 560 g ha<sup>-1</sup> or dicamba at 280 g ha<sup>-1</sup>.

None of the three auxin herbicides, regardless of application rate, nor glufosinate controlled Palmer amaranth greater than 80% (Table 2). Palmer amaranth can be present at densities of 100 or more plants m<sup>-2</sup> early in the season in nontreated cotton (Culpepper et al., 2006; Whitaker et al., 2011), and Palmer amaranth is competitive with cotton (Morgan et al., 2001; Rowland et al., 1999). In addition to high plant densities of Palmer amaranth, the competitiveness of the weed dictates the need for near perfect control.

We emphasize that these results are from single applications to weeds larger than the optimal size for treatment. Greater control would be expected if the Palmer amaranth had been smaller at application (Edwards et al., 2012; Voth et al., 2012). Similarly, greater control would be expected with a follow-up application of any of the four herbicides (Siebert et al., 2011). By intentionally delaying application until Palmer amaranth was 15 to 20 cm tall, we were better able to determine differences in efficacy of the herbicides and the effect of mixing auxin herbicides with glufosinate.

**Table 2. Control of Palmer amaranth, Benghal dayflower, and carpetweed 20 days after application of 2,4-DB, 2,4-D, and dicamba alone and mixed with glufosinate<sup>z</sup>**

Auxin herbicide	Auxin herbicide rate	Palmer amaranth		Benghal dayflower		Carpetweed	
		No glufosinate	+ glufosinate <sup>y</sup>	No glufosinate	+ glufosinate	No glufosinate	+ glufosinate
g ha <sup>-1</sup>		%					
No Auxin	---	--	74	--	68	--	100
2,4-DB	560	59	92 <sup>*w</sup>	60	78 <sup>*</sup>	59	100 <sup>*</sup>
2,4-DB	840	71	93 <sup>*</sup>	72	83 <sup>*</sup>	50	100 <sup>*</sup>
2,4-DB	1120	78	95 <sup>*</sup>	71	80 <sup>*</sup>	55	100 <sup>*</sup>
2,4-D	532	68	90 <sup>*</sup>	90	98 <sup>*</sup>	66	100 <sup>*</sup>
2,4-D	798	79	93 <sup>*</sup>	99	98	59	100 <sup>*</sup>
2,4-D	1064	80	97 <sup>*</sup>	98	99	63	100 <sup>*</sup>
Dicamba	280	59	89 <sup>*</sup>	69	97 <sup>*</sup>	55	100 <sup>*</sup>
Dicamba	560	76	92 <sup>*</sup>	84	94 <sup>*</sup>	58	100 <sup>*</sup>
Dicamba	1120	83	94 <sup>*</sup>	94	94	60	100 <sup>*</sup>
LSD (0.05)		9		9		13	

<sup>z</sup> Results for Palmer amaranth, tropical spiderwort, and carpetweed combined over 6, 1, and 1 locations, respectfully.

<sup>y</sup> Glufosinate applied 431 g ha<sup>-1</sup>.

<sup>w</sup> Means followed by an asterisk (\*) indicate the mixture of glufosinate plus auxin herbicide was more effective than the respective auxin herbicide and rate applied alone using Tukey's HSD at  $P \leq 0.05$  post-hoc pair-wise comparisons.

Compared with 74% control by glufosinate alone, auxin herbicides mixed with glufosinate increased Palmer amaranth control to 89 to 97% (Table 2). Control by all glufosinate/auxin combinations was greater than control by the auxin herbicides alone or glufosinate alone. Improved control of Palmer amaranth (Voth et al., 2012; York et al., 2012) and other weeds (Chahal and Johnson, 2012; Steckel et al., 2006) with mixtures of glufosinate and auxin herbicides has been observed in other studies.

**Benghal Dayflower.** Control of Benghal dayflower (*Commelina benghalensis* L.) in this study was as expected (Protsko, 2011a, 2011b). 2,4-D controlled Benghal dayflower 90 to 99% and was more effective than 2,4-DB or glufosinate (Table 2). Dicamba was as effective as 2,4-D when applied at 560 and 1120 g ha<sup>-1</sup>. Glufosinate applied alone controlled Benghal dayflower only 68%, but mixtures of glufosinate plus either 2,4-D or dicamba increased control to 94 to 99%. Control by mixtures of glufosinate plus 2,4-DB was greater than control by 2,4-DB or glufosinate alone, but combinations of glufosinate plus 2,4-DB were less effective than mixtures of glufosinate plus either 2,4-D or dicamba.

**Carpetweed.** Auxin herbicides controlled carpetweed (*Mollugo verticillata* L.) only 50 to 66% re-

gardless of product or rate used (Table 2). Glufosinate completely controlled carpetweed when applied alone or in combination with any of the auxin herbicides.

**Morningglory.** Regardless of rate, glufosinate and each auxin herbicide controlled morningglory species (*Ipomoea* spp.) completely (Table 3). Complete control also was obtained with all glufosinate plus auxin herbicide combinations. Auxin herbicides and glufosinate are expected to be effective on *Ipomoea* morningglory species (Corbett et al., 2004; Protsko, 2011a, 2011b).

**Broadleaf Signalgrass and Texas Millet.** Auxin herbicides did not control the two annual grass species: Texas millet (*Panicum texanum* [Buckl.] R. Webster) and broadleaf signalgrass (*Brachiaria platyphylla* [Nash] R. Webster) (Table 3). However, glufosinate controlled these grasses 89 to 90%. Texas millet control by mixtures of glufosinate plus dicamba was similar to control by glufosinate alone. In contrast, both 2,4-D and 2,4-DB mixed with glufosinate reduced Texas millet control 9 to 21%. Neither dicamba nor 2,4-DB mixed with glufosinate adversely affected broadleaf signalgrass control by glufosinate, but control with glufosinate plus 2,4-D was less than control by glufosinate alone in two of the three combinations.

Table 3. Control of morningglory, broadleaf signalgrass, and Texas millet 20 days after application of 2,4-DB, 2,4-D, and dicamba alone and mixed with glufosinate<sup>z</sup>

Auxin herbicide	Auxin herbicide rate	Morningglory <sup>y</sup>		Broadleaf signalgrass		Texas millet	
		No glufosinate	+ glufosinate <sup>x</sup>	No glufosinate	+ glufosinate	No glufosinate	+ glufosinate
	g ha <sup>-1</sup>	%					
No Auxin	---	--	100	--	89	--	90*
2,4-DB	560	100	100	0	95 <sup>*w</sup>	0	78*
2,4-DB	840	100	100	0	95*	0	70*
2,4-DB	1120	100	100	0	90*	0	69*
2,4-D	532	100	100	0	81*	0	81*
2,4-D	798	100	100	0	85*	0	81*
2,4-D	1064	100	100	0	82*	0	70*
Dicamba	280	100	100	0	90*	0	91*
Dicamba	560	100	100	0	89*	0	94*
Dicamba	1120	100	100	0	84*	0	95*
LSD (0.05)		NS		7		9	

<sup>z</sup> Results for morningglory, broadleaf signalgrass, and Texas millet combined over 2, 2, and 1 locations, respectively.

<sup>y</sup> Results for morningglory combined over one location with pitted morningglory and one with entireleaf morningglory..

<sup>x</sup> Glufosinate applied 431 g ha<sup>-1</sup>.

<sup>w</sup> Means followed by an asterisk (\*) indicate the mixture of glufosinate plus auxin herbicide was more effective than the respective auxin herbicide and rate applied alone using Tukey's HSD at  $P \leq 0.05$  post hoc pair-wise comparisons.

Previously published research shows that 2,4-D and 2,4-DB can reduce control of grassy weeds when these auxins are mixed with cyclohexanedione and aryloxyphenoxy propionate herbicides (Blackshaw et al., 2006; Mueller et al., 1989; York et al., 1993). Mixtures of glyphosate plus auxin herbicides usually have been additive, or sometimes synergistic, on dicot species (Chahal and Johnson, 2012; Culpepper et al., 2001; Jordan et al., 1997; Wehtje and Walker, 1997). Varying results have been reported with mixtures of glyphosate plus auxin herbicides applied to grassy weeds. 2,4-DB mixed with glyphosate had no effect on control of large crabgrass (Culpepper et al., 2001) or barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] (Jordan et al., 1997). 2,4-D mixed with glyphosate reduced control of johnsongrass, quackgrass, wheat, barley, and wild oat (O'Donovan and O'Sullivan, 1982). Dicamba and 2,4-D mixed with glyphosate reduced control of johnsongrass (Flint and Barrett, 1989).

Research with mixtures of auxin herbicides and glufosinate is much more limited. Dicamba and 2,4-D mixed with glufosinate generally have increased control of horseweed [*Conyza canadensis* (L.) Cronq.], common lambsquarters (*Chenopodium album* L.), and Palmer amaranth (Chahal and Johnson, 2012; Steckel et al., 2006; Voth et al., 2012; York et al., 2012). However, Botha et al. (2012) reported antagonism with dicamba plus reduced rates of glufosinate applied to Palmer amaranth. No results have been published on grass weed control by mixtures of glufosinate plus 2,4-D, 2,4-DB, or dicamba. However, quinclorac and triclopyr are auxin herbicides, and Lanclos et al. (2002) reported antagonism on barnyardgrass and broadleaf signalgrass with mixtures of glufosinate plus quinclorac or triclopyr.

New technologies allowing topical application of auxin herbicides to cotton will provide additional tools desperately needed by cotton growers to manage glyphosate-resistant weeds. However, auxin herbicides applied alone likely will not adequately control glyphosate-resistant Palmer amaranth. Glufosinate/auxin combinations will more effectively control Palmer amaranth and a broader spectrum of dicot weeds as compared to either of these chemistries applied alone. Additionally, the use of glufosinate/auxin combinations can potentially extend the useful life of both herbicides and technologies. Our results with glufosinate/auxin combinations on Texas millet and broadleaf signalgrass indicate the need for more research to better understand potential problems with the mixtures.

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## **Controlling Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Cotton with Resistance to Glyphosate, 2,4-D, and Glufosinate**

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## Controlling Glyphosate-Resistant Palmer Amaranth (*Amaranthus palmeri*) in Cotton with Resistance to Glyphosate, 2,4-D, and Glufosinate

Rand M. Merchant, A. Stanley Culpepper, Peter M. Eure, John S. Richburg, and L. Bo Braxton\*

Field experiments were conducted in Macon County, Georgia, during 2010 and 2011 to determine the impact of new herbicide-resistant cotton and respective herbicide systems on the control of glyphosate-resistant Palmer amaranth. Sequential POST applications of 2,4-D or glufosinate followed by diuron plus MSMA directed at layby (late POST-directed) controlled Palmer amaranth 62 to 79% and 46 to 49% at harvest when the initial application was made to 8- or 18-cm-tall Palmer amaranth, in separate trials, respectively. Mixtures of glufosinate plus 2,4-D applied sequentially followed by the layby controlled Palmer amaranth 95 to 97% regardless of Palmer amaranth height. Mixing glyphosate with 2,4-D improved control beyond that observed with 2,4-D alone, but control was still only 79 to 86% at harvest depending on 2,4-D rate. Sequential applications of glyphosate plus 2,4-D controlled Palmer amaranth 95 to 96% following the use of either pendimethalin or fomesafen. Seed cotton yield was at least 30% higher with 2,4-D plus glufosinate systems compared to systems with either herbicide alone. The addition of pendimethalin and/or fomesafen PRE did not improve Palmer amaranth control or yields when glufosinate plus 2,4-D were applied sequentially followed by the layby. The addition of these residual herbicides improved at harvest control (87 to 96%) when followed by sequential applications of 2,4-D or 2,4-D plus glyphosate; yields from these systems were similar to those with glufosinate plus 2,4-D. Comparison of 2,4-D and 2,4-DB treatments confirmed that 2,4-D is a more effective option for the control of Palmer amaranth. Results from these experiments suggest cotton with resistance to glufosinate, glyphosate, and 2,4-D will improve Palmer amaranth management. At-plant residual herbicides should be recommended for consistent performance of all 2,4-D systems across environments, although cotton with resistance to glyphosate, glufosinate, and 2,4-D will allow greater flexibility in selecting PRE herbicide(s), which should reduce input costs, carryover concerns, and crop injury when compared to current systems.

**Nomenclature:** 2,4-D; 2,4-DB; diuron; glufosinate; glyphosate; MSMA; Palmer amaranth, *Amaranthus palmeri* (S.) Wats. AMAPA; cotton, *Gossypium hirsutum* L.

**Key words:** Resistance management, tank mixtures, sequential herbicide applications.

Experimentos de campo fueron realizados en el condado Macon, Georgia, durante 2010 y 2011 para determinar el impacto de nuevos sistemas de algodón resistentes a herbicidas y sus respectivos herbicidas en el control de *Amaranthus palmeri* resistente a glyphosate. Aplicaciones secuenciales POST de 2,4-D o glufosinate seguidas de diuron más MSMA dirigidas a la base del cultivo (aplicaciones POST dirigidas tarde en el ciclo de crecimiento) controlaron *A. palmeri* 62 a 79% y 46 a 49% al momento de la cosecha cuando la aplicación inicial se hizo a *A. palmeri* de 8 a 18 cm de altura, en estudios independientes, respectivamente. Mezclas de glufosinate más 2,4-D aplicados secuencialmente seguidos por la aplicación dirigida controlaron *A. palmeri* 95 a 97% sin importar la altura de la maleza. El mezclar glyphosate con 2,4-D mejoró el control más allá del control observado con 2,4-D solo, pero aún así el control fue solamente 79 a 86% al momento de la cosecha, dependiendo de la dosis de 2,4-D. Aplicaciones secuenciales de glyphosate más 2,4-D controlaron *A. palmeri* 95 a 96% cuando se usaron después de aplicaciones de pendimethalin o fomesafen. El rendimiento de semilla del algodón fue al menos 30% mayor en sistemas con 2,4-D más glufosinate en comparación con los sistemas que tuvieron solamente aplicaciones de cualquiera de estos dos herbicidas solos. La adición de pendimethalin y/o fomesafen PRE no mejoró el control de *A. palmeri* ni los rendimientos cuando se realizaron aplicaciones secuenciales de glufosinate más 2,4-D seguidas por aplicaciones dirigidas. La adición de estos herbicidas residuales mejoró el control al momento de la cosecha (87 a 96%) cuando fueron seguidos de aplicaciones secuenciales de 2,4-D o 2,4-D más glyphosate. Los rendimientos de estos sistemas fueron similares a los de glufosinate más 2,4-D. Comparaciones entre tratamientos de 2,4-D y 2,4-DB confirmaron que 2,4-D es una opción más efectiva para el control de *A. palmeri*. Los resultados de estos experimentos

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sugieren que el algodón con resistencia a glufosinate, glyphosate, y 2,4-D mejorará el manejo de *A. palmeri*. El uso de herbicidas residuales debería ser recomendado para promover un desempeño consistente de todos los sistemas con 2,4-D en diferentes ambientes, aunque el algodón con resistencia a glyphosate, glufosinate, y 2,4-D permitirá una mayor flexibilidad en la selección de herbicidas PRE, lo cual podría reducir el costo en insumos, las preocupaciones por limitaciones en la rotación de cultivos debido a larga residualidad, y el riesgo de daño del cultivo, en comparación con los sistemas actuales.

First confirmed in 2004, glyphosate-resistant Palmer amaranth remains the primary weed of concern for cotton producers (Culpepper et al. 2006; Culpepper et al. 2010; Gaines et al. 2011; Whitaker et al. 2011a, 2011b). Efforts to control this pest have become more successful, but remain challenging and costly (Ford et al. 2011; Neve et al. 2011; Price et al. 2011). A grower survey conducted in 2010 reported that Georgia growers are spending \$168 ha<sup>-1</sup> on herbicides for the control of glyphosate-resistant Palmer amaranth, 2.5 times more herbicide active ingredient than they applied prior to resistance confirmation (Sosnoskie and Culpepper 2012). Use of residual herbicides (acetochlor, diuron, flumioxazin, fomesafen, pendimethalin, trifluralin, and S-metolachlor) applied throughout the crop as well as use of paraquat for preplant burndown and glufosinate for topical in-crop applications have increased. In conjunction with increased glufosinate use has been the adoption of cotton cultivars resistant to topical applications of glufosinate; increasing from 0% of Georgia's hectares in 2004 up to 49% of the hectares during 2012 (U.S. Department of Agriculture [USDA] 2004, 2012). Even after an aggressive herbicide system, 92% of Georgia growers are hand weeding 52% of the cotton crop at an average cost of \$60 ha<sup>-1</sup> for each hand-weeded hectare. Loss of conservation tillage is also occurring as growers adopt both primary and secondary tillage methods to aid in the battle against glyphosate-resistant Palmer amaranth (Sosnoskie and Culpepper 2012).

Agricultural biotechnology companies are developing new technologies that will increase the portfolio of herbicide-resistant crops. Herbicides labeled for use in these crops may provide effective options for the control of Palmer amaranth with resistance to currently used herbicides. One such technology will be cotton resistant to preplant or topical applications of 2,4-D (Braxton et al. 2010). 2,4-dichlorophenoxyacetic acid was the first selective herbicide widely used in agriculture (Peterson 1967). Much research has quantified its effective-

ness and limitations as a broadleaf herbicide in the decades since its discovery (Colby 1967; Migo et al. 1986; Triplett and Lytle 1972). Although 2,4-D is a member of the synthetic auxin family of herbicides, its site of action is currently unknown. Application of growth regulators, such as 2,4-D, induces an imbalance in phytohormone levels that causes epinasty of leaf stems and leaves and results in necrosis of meristematic tissue (Jursik et al. 2011). Synthetic auxins can be used for effective control of problematic broadleaves, such as common cocklebur (*Xanthium strumarium* L.), sicklepod (*Senna obtusifolia* L.), Palmer amaranth, and morningglory spp. (*Ipomoea* spp.) (Ferrell and Witt 2002; Lancaster et al. 2005; Norsworthy et al. 2008).

Cotton resistance to 2,4-D is conferred by the insertion of a gene that codes for an aryloxyalkanoate dioxygenase enzyme (Wright et al. 2010). Plants transformed to include this gene can metabolize certain auxin herbicides, including 2,4-D, to a nonlethal form (Richburg et al. 2012). The availability of cotton resistant to 2,4-D could increase POST herbicide options available to growers; especially when considering these 2,4-D-resistant cultivars will also be resistant to topical applications of glyphosate and glufosinate (Braxton et al. 2010). The objective of this study was to determine the most effective weed management system for the control of glyphosate-resistant Palmer amaranth in glyphosate, 2,4-D, and glufosinate-resistant cotton.

## Materials and Methods

Two experiments were each conducted twice in Macon County, GA, during 2010 and 2011 for a total of four site-years. Macon County was chosen for each site because the population of Palmer amaranth is highly glyphosate resistant and because the crop is grown under dryland conditions usually offering extremely stressful environments (Culpepper et al. 2006). The aad-12 gene is the transformation conferring 2,4-D resistance in cotton that

will be commercialized. However, because of limited seed availability, cotton with the *aad-1* gene, which also confers 2,4-D resistance, was used in these studies (Dow Agro Sciences; Indianapolis, IN). The *aad-1* gene has the ability to cleave members of the aryloxyphenoxy propionic acid family of grass-selective herbicides and is also able to inactivate 2,4-D, whereas the *aad-12* gene is more effective at deactivating 2,4-D due to greater *in vitro* activity (Wright et al. 2010). Seed was planted across each study at 10 seed per meter of row spaced 91 cm apart with the use of a vacuum planter. Soil was conventionally prepared by rotary tiller early season with individual plots 3.6 m wide by 7.6 m in length having treatments replicated four times. The soil type was a Dothan loamy sand with 1.9 to 2.1% organic matter and a pH of 6.2 to 6.4.

**Methods Specific to the 2,4-D Experiment.** A factorial treatment design including three PRE herbicide options and three POST herbicide options was implemented. PRE options included no herbicide, pendimethalin (Prowl H<sub>2</sub>O; BASF, Research Triangle Park, NC) at 1120 g ai ha<sup>-1</sup>, or fomesafen (Reflex; Syngenta Crop Protection, Research Triangle Park, NC) at 280 g ai ha<sup>-1</sup>. POST options were two sequential applications of 2,4-D (Weedar 64; NuFarm, Burr Ridge, IL) at 1,120 g ae ha<sup>-1</sup>, 2,4-D at 1,120 g ha<sup>-1</sup> plus glyphosate (Roundup WeatherMax; Monsanto, St. Louis, MO) at 840 g ae ha<sup>-1</sup>, or 2,4-D at 1,120 g ha<sup>-1</sup> plus glufosinate at 542 g ai ha<sup>-1</sup> (Ignite; Bayer CropScience, Research Triangle Park, NC). Five additional treatments without PRE herbicide included sequential applications of (1) 2,4-D at 840 g ha<sup>-1</sup>; (2) 2,4-D 840 g ha<sup>-1</sup> plus glyphosate at 840 g ai ha<sup>-1</sup>; (3) 2,4-D at 840 g ha<sup>-1</sup> plus glufosinate 542 g ai ha<sup>-1</sup>; (4) glyphosate alone at 840 g ai ha<sup>-1</sup>, and (5) glufosinate alone at 542 g ai ha<sup>-1</sup>. PRE applications were made the day of planting, POST 1 applications were made when Palmer amaranth reached 8 cm in height when no herbicide was applied PRE, and POST 2 applications were made 15 d after the POST 1 application. Location was planted on May 1 in 2010 and May 11 in 2011.

**Methods Specific to the 2,4-D vs. 2,4-DB Experiment.** A factorial treatment arrangement having three PRE herbicide options and five POST options was conducted. The three PRE options included no PRE, pendimethalin alone, or pendi-

methalin plus fomesafen. POST options included sequential applications of (1) 2,4-D at 840 g ha<sup>-1</sup>, (2) 2,4-DB at 840 g ae ha<sup>-1</sup>, (3) 2,4-D + glufosinate at 471 g ha<sup>-1</sup>, (4) 2,4-DB + glufosinate at 471 g ha<sup>-1</sup>, and (5) glufosinate alone at 471 g ha<sup>-1</sup>. PRE applications were made the day of planting, POST 1 applications were made when Palmer amaranth reached 18 cm in height in plots not receiving a PRE herbicide, and POST 2 applications were made 15 d after the POST 1 application. Location was planted on May 1 in 2010 and June 16 in 2011.

**Methods Common to Both Experiments.** Layby-directed applications of diuron at 1,120 g ai ha<sup>-1</sup> (Direx; DuPont Crop Protection, Wilmington, DE) plus MSMA at 1680 g ai ha<sup>-1</sup> (MSMA 6 Plus; Drexel Chemical Company, Memphis, TN) plus Crop Oil at 2.3 L ha<sup>-1</sup> (AGRI-DEX; Helena Chemical Company, Collierville, TN) were applied to all herbicide systems just prior to cotton canopy closure. Nontreated checks did not receive post-directed treatments. All applications were made with a CO<sub>2</sub>-pressurized backpack sprayer equipped with 11002 DG flat-fan nozzles calibrated to deliver 140 L ha<sup>-1</sup> at 165 kPa. No adjuvants were included with any PRE or POST application and a nontreated control was included for comparison. Insect control, fertilization, and defoliation practices were standard for dryland production in middle Georgia (Collins and Whitaker 2012).

Cotton plant heights were taken at layby and or at harvest by measuring the height of 20 plants per plot randomly. Cotton was harvested with a spindle picker modified for small-plot harvesting in November. Visual estimates of Palmer amaranth control were made prior to each herbicide application and at harvest using a visual scale of 0–100 with 0 = no control and 100 = complete plant death (Frans et al. 1986) Cotton stand was not influenced by treatments (data not shown) and seed cotton yield differences followed closely with late-season Palmer amaranth control, suggesting visual crop response had little impact on cotton yield. Palmer amaranth densities were obtained by counting all plants present between the two center rows of each plot following the layby application. There were no significant interactions between year and treatment, therefore data were combined over locations within experiments and analyzed with the use of PROC Mixed of SAS (SAS 9.2; SAS Institute, Cary, NC).

Site and replication were considered random effects, and treatments were considered fixed effects. Means were separated with the use of Fisher's LSD at  $P > 0.05$ . Treatments arranged as a factorial in the 2,4-D experiment were segregated and were analyzed as a factorial in PROC Mixed of SAS. This analysis did not alter the hierarchy of treatments compared to all treatments using a nonfactorial RCB design. Therefore comparisons were made with all treatments included.

## Results and Discussion

**2,4-D Experiment.** Glyphosate applied sequentially with no PRE treatment provided no control at layby or harvest (Table 1). Sequential applications of 2,4-D, with the first application applied to 8-cm Palmer amaranth, provided only 62 to 66% control at layby and control was less than that observed with sequential glufosinate applications (79%). Control was poor at harvest with both the 2,4-D or

glufosinate system after the layby was applied (62 to 79%), although the 2,4-D system with 1,120 g  $\text{ha}^{-1}$  was at least 11% more effective than when at the lower rate of 2,4-D or when glufosinate followed by a layby application was used. Mixing glyphosate with 2,4-D improved control beyond that observed with 2,4-D alone, but control was still only 79 to 86% at harvest depending on 2,4-D rate. Mixing glufosinate with 2,4-D controlled Palmer amaranth at least 95% throughout the season, both with and without a PRE treatment and regardless of 2,4-D rate used. The addition of pendimethalin or fomesafen PRE to sequential 2,4-D or glyphosate plus 2,4-D systems improved control to at least 93% at harvest with no differences between the two PRE options. Sequential applications of glufosinate plus 2,4-D following either PRE herbicide controlled Palmer amaranth 98 to 99% at harvest. Sequential applications of glyphosate plus 2,4-D following either PRE herbicide controlled Palmer amaranth 95 to 96% at harvest.

Table 1. Palmer amaranth control, cotton height, and seed cotton yield with POST systems including 2,4-D, glyphosate, and glufosinate.<sup>a</sup>

PRE herbicides		Sequential POST herbicides <sup>b,c</sup>	Palmer amaranth control		Cotton height prior to layby	Seed cotton yield
Pendimethalin	Fomesafen		At layby	At harvest		
g $\text{ha}^{-1}$	g $\text{ha}^{-1}$		%	%	cm	kg $\text{ha}^{-1}$
0	0	None	— <sup>d</sup>	—	18 e	0 g
0	0	2,4-D fb 2,4-D	66 ef	79 c	20 cde	960 cd
0	0	2,4-D + glyphosate fb 2,4-D + glyphosate	74 cd	86 bc	22 c	1,100 bc
0	0	2,4-D + glufosinate fb 2,4-D + glufosinate	97 a	95 a	27 b	1,360 a
1,120	0	2,4-D fb 2,4-D	86 b	93 ab	32 a	1,310 a
1,120	0	2,4-D + glyphosate fb 2,4-D + glyphosate	87 b	95 a	33 a	1,375 a
1,120	0	2,4-D + glufosinate fb 2,4-D + glufosinate	95 a	99 a	33 a	1,290 ab
0	280	2,4-D fb 2,4-D	89 b	95 a	34 a	1,355 a
0	280	2,4-D + glyphosate fb 2,4-D + glyphosate	98 a	96 a	35 a	1,350 a
0	280	2,4-D + glufosinate fb 2,4-D + glufosinate	99 a	98 a	34 a	1,350 a
0	0	2,4-D* fb 2,4-D*	62 f	68 d	19 de	715 e
0	0	Glyphosate fb glyphosate	0 g	0 f	20 cde	270 f
0	0	Glufosinate fb glufosinate	79 c	62 d	27 b	850 de
0	0	2,4-D* + glyphosate fb 2,4-D* + glyphosate	71 de	79 c	22 cd	980 cd
0	0	2,4-D* + glufosinate fb 2,4-D* + glufosinate	95 a	96 a	27 b	1,380 a

<sup>a</sup> Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test at  $P < 0.05$ . Data pooled over two locations. Diuron plus MSMA layby directed for all treatments except the nontreated control at time of canopy closure.

<sup>b</sup> Initial POST application made once Palmer amaranth reached 8 cm in height when no PRE was applied; sequential POST application made 15 d after the initial application. fb = followed by.

<sup>c</sup> 2,4-D applied at 1,120 g  $\text{ae ha}^{-1}$ , except when noted with an \*, indicating 2,4-D applied at 840 g  $\text{ha}^{-1}$ . Glyphosate and glufosinate applied at 840 and 471 g  $\text{ha}^{-1}$ , respectively.

<sup>d</sup> Data not included in the analysis, as they were assigned values of 0.

Table 2. Palmer amaranth control, cotton height, and seed cotton yield with POST systems including 2,4-D, 2,4-DB, and glufosinate.<sup>a</sup>

PRE herbicides			Palmer amaranth control		Palmer amaranth density after layby	Cotton height after layby	Seed cotton yield
Pendimethalin	Fomesafen	Sequential POST herbicides <sup>b,c</sup>	At layby	At harvest			
g ha <sup>-1</sup>	g ha <sup>-1</sup>		%	%	plants ha <sup>-1</sup>	cm	kg ha <sup>-1</sup>
0	0	None	— <sup>d</sup>	—	139,000 a	27 f	70 g
0	0	2,4-D fb 2,4-D	54 e	46 e	11,900 d	62 c	590 e
0	0	2,4-DB fb 2,4-DB	41 e	16 f	72,200 b	43 e	300 f
0	0	2,4-D + glufosinate fb 2,4-D + glufosinate	91 abc	97 a	0 h	71 ab	1,480 ab
0	0	2,4-DB + glufosinate fb 2,4-DB + glufosinate	88 bc	87 b	0 h	71 ab	1,390 b
0	0	Glufosinate fb glufosinate	69 d	49 e	9,400 e	64 c	710 de
1,120	0	None	10 g	0 g	23,800 c	57 d	110 g
1,120	0	2,4-D fb 2,4-D	86 c	87 b	300 h	71 ab	1,400 b
1,120	0	2,4-DB fb 2,4-DB	67 d	58 d	4,400 g	62 c	780 d
1,120	0	2,4-D + glufosinate fb 2,4-D + glufosinate	97 ab	98 a	0 h	73 ab	1,490 ab
1,120	0	2,4-DB + glufosinate fb 2,4-DB + glufosinate	98 ab	95 ab	0 h	72 ab	1,570 a
1,120	0	Glufosinate fb glufosinate	92 abc	91 ab	300 h	76 a	1,320 bc
1,120	280	None	66 d	19 f	7,500 f	74 ab	440 f
1,120	280	2,4-D fb 2,4-D	92 abc	93 ab	0 h	70 b	1,490 ab
1,120	280	2,4-DB fb 2,4-DB	86 c	77 c	600 h	75 ab	1,240 c
1,120	280	2,4-D + glufosinate fb 2,4-D + glufosinate	99 a	99 a	0 h	70 b	1,460 ab
1,120	280	2,4-DB + glufosinate fb 2,4-DB + glufosinate	98 ab	99 a	0 h	72 ab	1,500 ab
1,120	280	Glufosinate fb glufosinate	99 a	96 ab	0 h	74 ab	1,410 b

<sup>a</sup> Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test at  $P < 0.05$ . Data pooled over two locations. Diuron plus MSMA layby directed for all treatments except the nontreated control at time of canopy closure.

<sup>b</sup> Initial POST application made once Palmer amaranth reached 18 cm in height when no PRE was applied; sequential POST application made 15 d after the initial application.

<sup>c</sup> Glyphosate, glufosinate, 2,4-D, and 2,4-DB applied at 840, 471, 840, and 840 g ha<sup>-1</sup>, respectively. fb = followed by.

<sup>d</sup> Data not included in the analysis, as they were assigned values of 0.

Cotton plant heights were 22 to 48% taller in systems including a PRE herbicide compared to total POST systems at layby (Table 1). Early-season competition from Palmer amaranth has been well documented (Keeley and Thullen 1989; Morgan et al. 2001; Rowland et al. 1999) and even when making timely applications with effective POST herbicides, Palmer amaranth reduced cotton plant heights. Comparison of POST programs shows that cotton was 27 cm tall when glufosinate or glufosinate plus 2,4-D was applied and 18 to 22 cm when other herbicide systems were implemented.

Intense Palmer amaranth competition can reduce seed cotton yields and interfere with harvest efficiency (Fast et al. 2009; Morgan et al. 2001; Price et al. 2011). A Palmer amaranth population of 1 plant per 3 m of row can cause 13% yield loss; if the population increases to 10 plants per 3 m of

row, yield losses can increase to 57% (Fast et al. 2009). Greatest yields were achieved when Palmer amaranth was most effectively controlled. Palmer amaranth control at harvest ranged from 93 to 99% when systems included a PRE herbicide or when glufosinate plus 2,4-D was the POST option; these systems yielded from 1,290 to 1,380 kg ha<sup>-1</sup>. Total POST systems of 2,4-D at 840 g ha<sup>-1</sup>, 2,4-D at 1120 g ha<sup>-1</sup>, glufosinate, or glyphosate produced yields of 715, 960, 850, and 270 kg ha<sup>-1</sup>, respectively.

**2,4-D and 2,4-DB Experiment.** Delaying initial POST herbicide applications until Palmer amaranth reached 18 cm created a challenging weed control situation (Table 2). At layby, sequential 2,4-D (54%) or glufosinate (69%) programs provided unacceptable control and the addition of the layby had little effect with these two systems controlling Palmer amaranth only 46 to 49% at harvest. The



2,4-DB program was 13 and 30% less effective than comparative 2,4-D systems at layby or harvest, respectively. When applied in a timely manner, 2,4-DB is more effective than observed in this experiment, but Palmer amaranth control is still often not adequate (Grichar 1997). Mixtures of glufosinate and 2,4-D or 2,4-DB were more effective than any herbicide applied alone with control ranging from 88 to 91% at layby and 87 to 97% at harvest. The addition of pendimethalin PRE improved late-season control of sequential 2,4-D (87%), 2,4-DB (58%), and glufosinate (91%) systems, but did not improve control when POST options included an auxin mixed with glufosinate (95 to 98%). Fomesafen is effective in controlling Palmer amaranth (Everman et al., 2009), but the addition of fomesafen to pendimethalin PRE only improved control of the sequential 2,4-DB system.

Palmer amaranth densities following the layby were 139,000 plants ha<sup>-1</sup> in the nontreated control. Systems including an auxin plus glufosinate POST and the layby eliminated all Palmer amaranth plants, regardless of presence of a PRE herbicide. The only other two systems that eliminated Palmer amaranth populations included pendimethalin plus fomesafen PRE followed by sequential 2,4-D or glufosinate applications, and the layby.

Cotton heights were reflective of Palmer amaranth control. Cotton was at least 70 cm tall after the layby with all programs, including glufosinate plus an auxin POST, fomesafen PRE, or pendimethalin plus 2,4-D POST. Total POST programs with 2,4-D, 2,4-DB, or glufosinate resulted in shorter cotton ranging between 43 and 62 cm in height. Seed cotton yields were also reflective of Palmer amaranth populations present after the layby. Seed cotton yields of 1,390 to 1,570 kg ha<sup>-1</sup> were recorded from all systems including 2,4-D or 2,4-DB plus glufosinate and the layby, with or without a PRE herbicide. Other systems with similar yields exceeding 1,390 kg ha<sup>-1</sup> included pendimethalin or pendimethalin plus fomesafen followed by sequential 2,4-D or glufosinate applications and the layby. Direct comparison of auxin programs with and without PRE herbicides shows that yields were always higher with the 2,4-D-alone system as compared to the 2,4-DB-alone system.

Cotton technology with resistance to glyphosate, 2,4-D and glufosinate will improve grower flexibility and postemergence management of Palmer

amaranth. Numerous effective systems can be developed with this technology. In these studies, tank mixtures of glufosinate plus 2,4-D or systems containing both 2,4-D and glufosinate were among the most effective for controlling emerged Palmer amaranth. Additionally, at-plant residual herbicides allow for consistent performance of all 2,4-D systems when compared to current systems (Monks et al. 2012; Sosnoskie et al. 2011; Sosnoskie and Culpepper 2012).

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**Comments of the Natural Resources Defense Council on the October 31, 2016  
Proposed Registration Decision of Enlist Duo Herbicide**

**EPA-HQ-OPP-2016-0594  
December 1, 2016**

The Natural Resources Defense Council (NRDC) is a national, non-profit environmental organization of lawyers, scientists, and other professionals. NRDC presents these comments on behalf of our 1.3 million members and online activists. NRDC does not have any financial interest in the topic of these comments.

In November 2011, Dow AgroSciences (Dow) submitted an application for registration of Enlist Duo, an herbicide containing the active ingredients glyphosate dimethylammonium salt (glyphosate) and 2,4-dichlorophenoxyacetic acid choline salt (2,4-D), for use on corn and soybean crops that have been genetically engineered (GE) to be tolerant to glyphosate and 2,4-D (Enlist corn and soy). EPA subsequently issued a proposed registration decision for Enlist Duo, and NRDC submitted comments on the proposed decision. *See* EPA, Proposed Registration of Enlist Duo Herbicide (Apr. 30, 2014); NRDC, Comments of the Natural Resources Defense Council on Dow AgroSciences' Application to Register Enlist Duo Herbicide Containing the Choline Salt of 2,4-D and Glyphosate (June 30, 2014) [hereinafter NRDC, Enlist Duo Comments—Six States]. On October, 15 2014, EPA registered Enlist Duo for use on Enlist corn and soy in six states: Illinois, Indiana, Iowa, Ohio, South Dakota, and Wisconsin. *See* EPA, Notice of Pesticide Registration for Enlist Duo (October 15, 2014).

That same day, EPA also opened a thirty-day comment period on whether to register Enlist Duo for use in ten additional states: Arkansas, Kansas, Louisiana, Minnesota, Missouri, Mississippi, Nebraska, Oklahoma, Tennessee and North Dakota. *See* Memorandum from Dan Rosenblatt, Acting Director, Registration Division, US EPA/OSCPP/OPP, to Kathryn V.



Montague, Product Manager 23, Herbicide Branch, Registration Division, US EPA/OSCPP/OPP (Oct. 15, 2014). NRDC submitted comments in response to EPA’s proposal to register Enlist Duo for use on Enlist corn and soy in these ten additional states. *See* NRDC, Comments of the Natural Resources Defense Council on Dow AgroSciences’ Application to Register Enlist Duo Herbicide Containing the Choline Salt of 2,4-D and Glyphosate in Ten Additional States (Nov. 14, 2014) [hereinafter NRDC, Enlist Duo Comments—Ten States]. On March 31, 2015, EPA issued an amended decision registering Enlist Duo for use in nine additional states: Arkansas, Kansas, Louisiana, Minnesota, Missouri, Mississippi, Nebraska, Oklahoma, and North Dakota. *See* EPA, Notice of Pesticide Registration for Enlist Duo (October 15, 2014). *See* EPA, Decision to Amend Enlist Duo Herbicide Label to include additional states: Arkansas, Kansas, Louisiana, Minnesota, Missouri, Mississippi, Nebraska, Oklahoma, and North Dakota (Mar. 31, 2015).

NRDC and other groups brought legal challenges in the Ninth Circuit Court of Appeals to EPA’s registration decisions for Enlist Duo, and the Ninth Circuit ultimately remanded the registration to EPA. *See* Order, *NRDC v. EPA*, No. 14-73353, ECF No. 128 (Jan. 25, 2016). Thereafter, on October 27, 2016, EPA posted a public notice in the Federal Registrar announcing Dow’s application to register Enlist Duo on “[c]otton, gin byproducts and cotton, [and] delinted seed.” *See* 81 Fed. Reg. 208, Pesticide Product Registration; Receipt of Application for New Use (Oct. 27, 2016). On October 31, 2016, EPA issued a new decision “propos[ing] to maintain the previously approved uses of Enlist Duo on GE corn and soybeans in 15 states with no changes to the original registration, as amended, with the exception of adding 19 states to the label, and adding the new use of Enlist Duo on GE cotton in all 34 states.” EPA, Proposed Registration Decision of Enlist Duo Herbicide at 3 (Oct. 31, 2016) [hereinafter EPA, Proposed Registration Decision].

NRDC submits these comments in response to EPA's October 31, 2016 Proposed Registration Decision of Enlist Duo Herbicide, and also incorporates by reference its comments on EPA's previous proposed registration decisions for Enlist Duo, *see* NRDC, Enlist Duo Comments—Six States; NRDC, Enlist Duo Comments—Ten States, and all the documents cited in these three sets of comments.<sup>1</sup>

## I. INTRODUCTION

NRDC has serious concerns about the sustained (and likely expanded) use of glyphosate, as well as the expanded use of 2,4-D, that would follow EPA's registration of Enlist Duo for use on GE corn, GE soybeans, and GE cotton in thirty-four states.

### A. GLYPHOSATE

Glyphosate is an active ingredient<sup>2</sup> in herbicides that have been registered for many agricultural and non-agricultural uses.<sup>3</sup> EPA has approved the use of glyphosate-based herbicides on over 100 terrestrial food crops, including fruit, vegetable, and field crops.<sup>4</sup> The agency has also registered glyphosate-based herbicides for use in non-crop settings, often to achieve total

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<sup>1</sup> With the exception of some documents that are part of the Enlist Duo public dockets (EPA-HQ-OPP-2016-0594, EPA-HQ-OPP-2014-0195), studies cited by the EPA documents included in the Enlist Duo public dockets, and court documents, NRDC has put the documents cited in these comments onto a DVD sent via FedEx to EPA on December 1, 2016. NRDC requests that EPA consider all cited documents part of NRDC's comments.

<sup>2</sup> Glyphosate is an "active ingredient" used in pesticide products. *See* 7 U.S.C. § 136(a) (defining "active ingredient" to mean "an ingredient which will prevent, destroy, repel, or mitigate any pest"). The term "glyphosate" is, however, often used as a shorthand for glyphosate-based herbicides. *Cf. id.* § 136(u) (defining "pesticide" to include "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest").

<sup>3</sup> EPA, Glyphosate Final Work Plan (FWP): Registration Review Case No. 0178, at 2 (2009) [hereinafter 2009 Glyphosate Final Work Plan].

<sup>4</sup> *Id.*

vegetation control.<sup>5</sup> Non-crop areas to which glyphosate-based herbicides are applied include residential, industrial, forestry, greenhouse, ornamental, aquatic, and other sites.<sup>6</sup> When applied at lower rates, glyphosate also functions as a plant growth regulator.<sup>7</sup>

EPA registered its first glyphosate-based herbicide in 1974. As a non-selective herbicidal ingredient, glyphosate does not discriminate between target and non-target plant species. Because of its damage to crops, the use of glyphosate was initially limited. Since EPA reregistered glyphosate-based herbicides in 1993, however, the development of genetically-modified, glyphosate-resistant crops has facilitated a dramatic rise in the application of glyphosate-containing herbicides. Approximately 276 million pounds of glyphosate were applied in 2014—compared to about 18.7 million pounds in 1991.<sup>8</sup> Having experienced an over ten-fold increase since the years preceding its reregistration, glyphosate is now the most widely used pesticide active ingredient in the United States.<sup>9</sup>

The unprecedented increase in glyphosate use has adversely affected the North American monarch butterfly, *Danaus plexippus*, an iconic species that migrates through the United States

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<sup>5</sup> EPA, Reregistration Eligibility Decision Facts: Glyphosate, at 1 (1993) [hereinafter 1993 Glyphosate R.E.D. Facts].

<sup>6</sup> 2009 Glyphosate Final Work Plan, *supra* note 3, at 2; 1993 Glyphosate R.E.D. Facts, *supra* note 5, at 1.

<sup>7</sup> 1993 Glyphosate R.E.D. Facts, *supra* note 5, at 1.

<sup>8</sup> Compare EPA, Reregistration Eligibility Decision: Glyphosate, at 9 (1993) [hereinafter 1993 Glyphosate R.E.D.], with Charles M. Benbrook, *Trends in Glyphosate Herbicide Use in the United States and Globally*, *Envtl. Sci. Europe* 28:3, at 5 tbl.1 (2016); see also U.S. EPA, 2006-2007 Pesticide Market Estimates: Usage, at 14-15, [https://www.epa.gov/sites/production/files/2015-10/documents/market\\_estimates2007.pdf](https://www.epa.gov/sites/production/files/2015-10/documents/market_estimates2007.pdf) (last visited Nov. 22, 2016).

<sup>9</sup> See Benbrook, *supra* note 8, at 5 tbl.1 (2016); U.S. Geological Survey, *Glyphosate Found in Wastewater Discharged to Streams*, [http://toxics.usgs.gov/highlights/glyphosate\\_wastewater.html](http://toxics.usgs.gov/highlights/glyphosate_wastewater.html) (last visited Nov. 23, 2016) (noting that glyphosate is also the most widely used herbicide in the world).

as part of its annual life cycle. Over the last decade, there has been a sharp decline in the monarch population that traverses the United States and overwinters in Mexico.<sup>10</sup> By eliminating milkweed—the exclusive food source for monarch larvae—the pervasive use of glyphosate has contributed to the monarch’s decline. The decimation of milkweed, particularly from agricultural areas, has been associated with an 81% decrease in the production of monarchs in the Midwest and a 65% decrease in the size of the entire monarch population that overwintered in Mexico between 1999 and 2010. The population reached an all-time low in 2014 when the overwintering butterflies occupied only 0.67 hectares—down from a high of 18.19 hectares in 1997.<sup>11</sup>

In addition to contributing to a significant decline in the monarch population, glyphosate poses a serious risk to human health. In the more than two decades since EPA last completed a review of glyphosate’s health risks, significant new studies have been published addressing those

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<sup>10</sup> Monarch butterflies are found both east and west of the Rocky Mountains, although the western population is much smaller than its eastern counterpart. *See* Monarch Joint Venture, Monarch Migration, <http://monarchjointventure.org/monarch-biology/monarch-migration> (last visited Nov. 23, 2016). These comments focus on the eastern population of North American monarchs, because it is this population that traverses the thirty-four states where EPA has proposed to register Enlist Duo. *See id.*; Leonard I. Wassenaar & Keith A. Hobson, *Natal Origins of Migratory Monarch Butterflies at Wintering Colonies in Mexico: New Isotopic Evidence*, 95 Proc. Nat’l Acad. Sci. U.S. 15436, 15438 & fig.3 (1998); Ctr. for Food Safety, *Correlation Between Glyphosate Use and Monarch Migration Routes and Breeding*, <http://www.centerforfoodsafety.org/issues/304/pollinators-and-pesticides/map-of-monarch-migration-breeding-and-glyphosate-use#> (last visited Nov. 30, 2016).

<sup>11</sup> Monarch Watch, Monarch Population Status (Feb. 26, 2016), <http://monarchwatch.org/blog/2016/02/26/monarch-population-status-26/>. The area covered by overwintering monarchs is a proxy for the overwintering monarch population size. *See, e.g.*, Lincoln P. Brower et al., *Catastrophic Winter Storm Mortality of Monarch Butterflies in Mexico During January 2002*, in *The Monarch Butterfly: Biology and Conservation* 151, 162 (Karen S. Oberhauser & Michelle J. Solensky, eds.) (2002) (assuming density of approximately 50 million butterflies per hectare); Pollinator Health Task Force, National Strategy to Promote the Health of Honey Bees and Other Pollinators (May 19, 2015) (assuming an estimated density of 37.5 million butterflies per hectare); Brice X. Semmens, *Quasi-Extinction Risk and Population Targets for the Eastern, Migratory Population of Monarch Butterflies (Danaus Plexippus)*, Scientific Reports 6:23265, at 4 (2016).

risks. Among other things, based on a comprehensive evaluation of the relevant publicly-available literature, the World Health Organization's cancer evaluation arm, the International Agency for Research on Cancer (IARC), recently found that glyphosate is probably carcinogenic to humans.<sup>12</sup> Despite IARC's cancer-finding for glyphosate, EPA is proposing to register Enlist Duo without an updated risk assessment for the chemical. EPA's current glyphosate assessment is 23 years old, and over 3,000 studies have been published since that time, many of which provide a basis for much more stringent restrictions to address both cancer and non-cancer effects; these studies also contain emerging information on human exposures to glyphosate from widespread residues in common foods, including many foods eaten often by children. NRDC strongly objects to the registration of Enlist Duo without an updated comprehensive human health risk assessment for glyphosate that adequately addresses dietary exposures through foods, and vulnerable populations including pregnant women, infants and children, farmworkers and their families, and suburban communities next to treated fields.

EPA is currently reevaluating glyphosate to determine if it still meets the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) standard for registration. The final registration review process was scheduled to end in 2015,<sup>13</sup> but that deadline has come and gone without publication of even a preliminary risk assessment. EPA cannot properly find that Enlist Duo will not cause unreasonable adverse effects on human health or the environment, and

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<sup>12</sup> The World Health Organization issued this cancer finding shortly before EPA expanded Enlist Duo's initial registration to an additional nine states. NRDC and other concerned groups promptly notified EPA of this finding, asking the agency to reconsider its initial Enlist Duo registration and not to register Enlist Duo for use in additional states until it considered and addressed the cancer risk. *See* Ctr. for Food Safety et al., Letter to Gina McCarthy (Mar. 26, 2015). EPA ignored the new cancer finding and registered Enlist Duo for use in the nine additional states anyway, without explaining why it declined to evaluate the cancer risk.

<sup>13</sup> 2009 Glyphosate Final Work Plan, *supra* note 3, at 3.

therefore cannot make a final registration decision for Enlist Duo, until it has completed its updated assessment of glyphosate's effects on human health and the environment, and has provided for public notice and comment on that assessment.

## **B. 2,4-D**

2,4-D is a widely used herbicidal active ingredient with nearly 40 million pounds used on agricultural crops in 2011. Dermal contact, ingestion of contaminated food and water, and inhalation represent the major routes of human exposure. 2,4-D has been implicated in a number of adverse human health endpoints, including Non-Hodgkin lymphoma, decreased fertility, and higher rates of birth defects. In 2015, IARC classified 2,4-D as a "possible" human carcinogen (Group 2B) based largely on strong evidence in cellular studies that 2,4-D causes the type of cellular damage that can lead to cancer (oxidative stress and immunosuppression), as well as limited evidence of cancer in laboratory animal studies.<sup>14</sup>

Unfortunately, EPA's assessments of both the toxicity of 2,4-D and the relevant exposure pathways for Enlist Duo are critically flawed and fall short of what is necessary to protect human health. In addition, the agency's proposal to eliminate the ten-fold FQPA safety factor cannot be justified. With respect to carcinogenicity, EPA ignored IARC's recent cancer finding for 2,4-D, simply stating that "2,4-D has been classified as a Category D chemical, i.e., not classifiable as

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<sup>14</sup> IARC, IARC Monographs Vol. 113 – 2,4-Dichlorophenoxyacetic acid (2,4-D) and Some Organochlorine Insecticides (2016), *available at* <http://monographs.iarc.fr/ENG/Monographs/vol113/mono113-03.pdf> [hereinafter IARC 2,4-D Monograph]; *see also* Dana Loomis et al., Monograph Working Group, IARC, Carcinogenicity of Lindane, DDT, and 2,4-D (June 23, 2015); Press Release, IARC, World Health Org., IARC Monographs Evaluate DDT, Lindane, and 2,4-D (June 23, 2015), *available at* [http://www.iarc.fr/en/media-centre/pr/2015/pdfs/pr236\\_E.pdf](http://www.iarc.fr/en/media-centre/pr/2015/pdfs/pr236_E.pdf); IARC, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 113 (2016), <http://monographs.iarc.fr/ENG/Monographs/vol113/index.php> (last visited Nov. 23, 2016).

to human carcinogenicity. A quantitative cancer risk assessment is not required.”<sup>15</sup> But this classification was made two decades ago, back in 1996.<sup>16</sup> In light of IARC’s recent cancer finding, and the post-1996 studies that contributed to this finding, it is inappropriate for EPA to rely on its outdated carcinogenicity classification for 2,4-D in registering Enlist Duo.

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In evaluating Dow’s application to register Enlist Duo for use on GE corn, GE soybean, and GE cotton, EPA is required to determine whether registration would lead to unreasonable adverse impacts on human health and the environment. EPA has failed to undertake the full range of analyses necessary to make this determination. As discussed in these comments, the agency must consider the substantial adverse impacts that Enlist Duo will have on monarch butterflies, insofar as both glyphosate and 2,4-D destroy milkweed, which is critical to the monarch life cycle. EPA must also expand its assessment of the serious risks that Enlist Duo may pose to human health. Among other things, EPA must complete an updated assessment of glyphosate’s human health risks, including cancer risk and chronic dietary risk, rather than rely on an assessment that is over two decades old. In addition, there are multiple flaws in the agency’s human health risk assessment for 2,4-D, including but not limited to failure to consider new evidence of cancer risk.

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<sup>15</sup> EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide Tolerant Cotton, at 5, 35 (2016), *available at* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0594-0009>; *accord* EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean, at 4, 37 (2013).

<sup>16</sup> EPA, Reregistration Eligibility Decision for 2,4-D, at 19 (2005) [hereinafter 2005 2,4-D R.E.D.] (“2,4-D has been classified as a Category D chemical (i.e., not classifiable as to human carcinogenicity), by the EPA/OPP Cancer Peer Review Committee in 1996.”).



## II. LEGAL FRAMEWORK

Under FIFRA, any new pesticide must be “registered” with EPA before it can be distributed, sold, or used in the United States. 7 U.S.C. § 136a(a); *Pollinator Stewardship Council*, 800 F.3d at 1177. A pesticide includes “any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest,” including any “weed,” and thus includes chemicals commonly known as herbicides (or weed killers). 7 U.S.C. § 136(t), (u). Registration under FIFRA is either unconditional or conditional. *Hardin v. Jackson*, 625 F.3d 739, 740 (D.C. Cir. 2010) (citing 7 U.S.C. §§ 136a(c)(5), 136a(c)(7)).

As for unconditional registration, FIFRA authorizes EPA to register a pesticide only upon determining that the pesticide “will perform its intended function without unreasonable adverse effects on the environment,” and that “when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.” *Id.* § 136a(c)(5)(C), (D); *accord* 40 C.F.R. § 152.112(e). The statute defines “unreasonable adverse effects on the environment” to include “any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide.” 7 U.S.C. § 136(bb).

Before registering a pesticide, EPA is required to “review . . . all relevant data in the possession of the Agency” and determine “that no additional data are necessary to make the determinations required by FIFRA sec. 3(c)(5) [7 U.S.C. § 136a(c)(5)].” 40 C.F.R. § 152.112(b), (c); *accord Pollinator Stewardship Council*, 800 F.3d at 1183. EPA’s regulations require the agency to consider information “sufficient to evaluate the potential of the [pesticide] product to cause unreasonable adverse effects on man and the environment.” 40 C.F.R. § 158.75.

In addition, EPA “may conditionally register . . . a pesticide if the Administrator determines that

(i) the pesticide and proposed use are identical or substantially similar to any currently registered pesticide and use thereof, or differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment, and (ii) approving the registration or amendment in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment.

7 U.S.C. § 136a(c)(7)(A); *see also* 40 C.F.R. § 152.113. “An applicant seeking conditional registration . . . shall submit such data as would be required to obtain registration of a similar pesticide under paragraph 5 [§ 136a(c)(5), setting forth the requirements for unconditional registration].” *Id.* “If the applicant is unable to submit an item of data because it has not yet been generated,” however, “the Administrator may register or amend the registration of the pesticide under such conditions as will require the submission of such data not later than the time such data are required to be submitted with respect to similar pesticides already registered under this subchapter.” *Id.*

The regulations for conditional registration further provide that EPA “may approve an application for registration . . . of a pesticide product, each of whose active ingredients is contained in one or more other registered pesticide products,” only if, among other things, the agency has determined that “(1) [i]t possesses all data necessary to make the determinations required by FIFRA sec. 3(c)(7)(A) . . . with respect to the pesticide product which is the subject of the application (including, at a minimum, data needed to characterize any incremental risk that would result from approval of the application),” and “(2) [a]pproval of the application would not significantly increase the risk of any unreasonable adverse effect on the environment.” 40 C.F.R. § 152.113(a). Notwithstanding the satisfaction of these requirements, EPA “will not approve the conditional registration of any pesticide” unless the agency has determined that “the applicant’s

product and its proposed use are identical or substantially similar to a currently registered pesticide and use, or that the pesticide and its proposed use differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment.” *Id.*

§ 152.113(b).

After a new pesticide has been registered, EPA must periodically review that registration to make sure it is still considered safe in light of new science. *See* 7 U.S.C. § 136a(g). EPA must complete its review of each existing pesticide registration by either October 2022 or within 15 years after the date on which a pesticide containing a new active ingredient is first registered, whichever is later. *Id.* § 136a(g)(1)(A)(iii). Thereafter, EPA is required to conduct subsequent reviews of each pesticide registration every fifteen years. *Id.* § 136a(g)(1)(A)(iv).

EPA has explained that “Registration Review is a lengthy process that may take many years to complete” and that “the Agency’s policy is to continue to make registration determinations for new actions during this process.” EPA, Response to Public Comments Received Regarding New Uses of Enlist Duo on Corn and Soybeans, at 25 (Oct. 14, 2014) [hereinafter EPA, 2014 Response to Public Comments]. EPA has further explained that, despite the concurrent progress of any registration reviews, “[p]roposed new registrations are held to the most current data requirements and up-to-date risk assessment practices and must meet the FIFRA no unreasonable adverse effects standard to be registered.” *Id.*

In addition to the required registration process (for new pesticides) and registration reviews (for existing pesticides), EPA has the authority to conduct interim administrative reviews of pesticide registrations if there is “significant evidence raising prudent concerns of unreasonable adverse risk to man or to the environment.” 7 U.S.C. § 136a(c)(8). To this end, EPA has promulgated regulations providing for Special Review of registered pesticides “to help

the Agency determine whether to initiate procedures to cancel, deny, or reclassify registration of a pesticide product because uses of that product may cause unreasonable adverse effects on the environment.” 40 C.F.R. § 154.1.

### III. DISCUSSION

#### A. **Enlist Duo is a new herbicide combining glyphosate and 2,4-D that will perpetuate glyphosate use and increase 2,4-D use**

Enlist Duo is an herbicide that contains the active ingredients glyphosate and 2,4-D.<sup>17</sup> It is designed for use on Enlist Duo-resistant corn, soybean, and cotton crops.<sup>18</sup> In other words, Enlist Duo is specifically meant for application to crops that are genetically engineered to resist the herbicide, so that it can be sprayed later in the growing season and in greater amounts, to kill weeds without killing the crops.<sup>19</sup> The use of 2,4-D on herbicide-resistant crops is new.<sup>20</sup> Glyphosate has been used on herbicide-resistant crops for two decades, often under the trade name Roundup (a line of glyphosate-based herbicides developed by Monsanto).

Widespread use of glyphosate has spawned a burgeoning problem of weeds that have developed glyphosate resistance. The U.S. Department of Agriculture has recognized that the “nearly exclusive use of glyphosate over the past fifteen years led to the selection of glyphosate-resistant . . . weeds, weeds that could survive an application of the herbicide that once would kill

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<sup>17</sup> EPA, Proposed Registration Decision, at 1.

<sup>18</sup> *Id.*

<sup>19</sup> *Id.* at 27; Bill Phillips II, EPA, Discussion of the Benefits for Enlist Duo Use on Herbicide Resistant Soybeans and Corn, at 3-4, (Apr. 30, 2014), *available at* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0195-0011>; Ctr. for Food Safety, Comments to USDA APHIS on Dow AgroSciences LLC; Draft Environmental Impact Statement for Determination of Nonregulated Status of Herbicide Resistant Corn and Soybeans, at 14 (Mar. 11, 2014) [hereinafter 2014 CFS Comments].

<sup>20</sup> EPA, Final Registration Decision for Enlist Duo Herbicide, at 1 (Oct. 14, 2014); EPA, Proposed Registration Decision, at 2.

earlier generations.”<sup>21</sup> In response to a three-year survey in thirty-one states, forty-nine percent of farmers reported having glyphosate-resistant weeds on their farms in 2012.<sup>22</sup> In its comments to EPA in support of registering Enlist Duo, Dow stated that the problem of “increasing prevalence of glyphosate-resistant” weeds is “rapidly getting worse.”<sup>23</sup> And according to EPA, “resistance to glyphosate and other herbicides has become a significant economic and pest management issue to growers.”<sup>24</sup>

Thus, under current use patterns, glyphosate is rapidly becoming ineffective. EPA has concluded that “[t]he continued viability of the glyphosate . . . technology is widely predicated on the containment of currently resistant weed biotype populations and the delay of any future resistant weed biotype population development.”<sup>25</sup> And Dow has asserted that without new pesticides like Enlist Duo “to address problems with glyphosate-resistant weeds, U.S. growers will be forced to revert to earlier cultural practices” that did not rely so heavily on glyphosate.<sup>26</sup>

Enlist Duo is intended to be “another tool that could prolong the viability of the glyphosate herbicide technology” by incorporating two herbicides with different mechanisms of

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<sup>21</sup> Animal and Plant Health Inspection Service (APHIS), U.S. Dep’t of Agric., Final Environmental Impact Statement for Dow AgroSciences Petitions (09-233-01p, 09-349-01p, and 11-234-01p) for Determinations of Nonregulated Status for 2,4-D-Resistant Corn and Soybean Varieties, at iii (2014), *available at* [https://www.aphis.usda.gov/brs/aphisdocs/24d\\_feis.pdf](https://www.aphis.usda.gov/brs/aphisdocs/24d_feis.pdf).

<sup>22</sup> Ctr. for Food Safety, Comments on Registration Review of the Herbicide 2,4-D, at 5 (Feb. 12, 2013) [hereinafter CFS 2013 Comments].

<sup>23</sup> Dow, Comments of Dow AgroSciences LLC, EPA Docket No.: EPA-HQ-OPP-2012-0012, at 1, 4 (June 22, 2012); *see also* Ctr. for Food Safety, Comments to EPA on Notice of Receipt of Application to Register New Uses of 2,4-D on Enlist AAD-1 Corn and Soybean, at 14 (June 22, 2012) [hereinafter CFS 2012 Comments].

<sup>24</sup> EPA, Proposed Registration Decision, at 27.

<sup>25</sup> Phillips, *supra* note 19, at 8.

<sup>26</sup> Dow, *supra* note 23, at 4.

action.<sup>27</sup> In other words, the combination of chemicals in Enlist Duo is meant to kill weeds that would resist glyphosate alone.<sup>28</sup>

Thus, at a minimum, Enlist Duo will facilitate the continued use of glyphosate at levels well above what would otherwise be expected, given glyphosate's declining efficacy.<sup>29</sup> EPA did not assess how registration of Enlist Duo would affect total herbicide loading in the environment.<sup>30</sup> Rather, EPA noted that the analysis was "difficult" and the agency could reach no conclusions.<sup>31</sup> Nonetheless, EPA assumed (without citing any evidence) that Enlist Duo will not increase total glyphosate use, because it expects Enlist Duo to substitute for existing uses of other glyphosate-containing pesticides.<sup>32</sup>

If Enlist Duo works as designed, however, it has the potential to expand glyphosate use by enticing additional growers to switch from conventional crops to herbicide-resistant Enlist Duo crops. Despite tremendous growth in the proportions of corn, soybean, and cotton crops that are herbicide-resistant, there is still room for further increases.<sup>33</sup> Enlist Duo is designed to appeal to growers by giving them an herbicide option that can overcome glyphosate-resistant weeds and

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<sup>27</sup> Phillips, *supra* note 19, at 3, 9.

<sup>28</sup> *Id.* at 2-4, 8.

<sup>29</sup> *Id.* at 3.

<sup>30</sup> *Id.* at 9.

<sup>31</sup> *Id.*

<sup>32</sup> EPA, 2014 Response to Public Comments, at 32.

<sup>33</sup> Econ. Research Serv., USDA, Recent Trends in GE Adoption, <https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx> (last visited Nov. 29, 2016) (as of 2016, 89% of corn and cotton crops, and 94% of soybean crops, were glyphosate-resistant).

be used over a longer portion of the growing season.<sup>34</sup>

As for 2,4-D, EPA recognizes that registration of Enlist Duo makes it “likely that 2,4-D use will increase.”<sup>35</sup> The Department of Agriculture agreed, predicting that approval of Enlist Duo will cause a two- to six-fold increase in the overall use of 2,4-D.<sup>36</sup> The Department of Agriculture further noted that approval of Enlist Duo will allow 2,4-D “to be used over a wider part of the growing season.”<sup>37</sup> More specifically, EPA has acknowledged:

Current registered uses of non-choline 2,4-D in corn allow for over-the-top broadcast applications only up to 8 inches tall which would be increased to up to 48 inches tall with GE 2,4-D tolerant corn. Similarly, the currently registered use of non-choline 2,4-D in soybeans allows pre-plant applications only, however new uses of 2,4-D choline salt would expand uses to include over-the top broadcast applications to GE soybeans. Currently registered uses of non-choline 2,4-D in cotton allow for a preplant application or a fall postharvest broadcast or spot treatment. The proposed new use of 2,4-D choline salt in GE cotton would allow Enlist Duo to be applied post-emergence during the growing season.<sup>38</sup>

EPA needs to take into consideration the continued (and possibly expanded) use of glyphosate, as well as the expanded use of 2,4-D, when assessing the risks that Enlist Duo would pose to monarchs and human health.

## **B. Enlist Duo Poses a Serious Risk to Monarch Butterflies**

Use of Enlist Duo poses a significant risk to monarch butterflies because both glyphosate and 2,4-D kill milkweed, which provides essential monarch food and habitat.<sup>39</sup>

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<sup>34</sup> Phillips, *supra* note 19, at 2-4.

<sup>35</sup> *Id.* at 9.

<sup>36</sup> APHIS, *supra* note 21, at x.

<sup>37</sup> *Id.*

<sup>38</sup> EPA, Proposed Registration Decision, *supra*, at 27.

<sup>39</sup> EPA, Proposed Label for Enlist Duo (Oct. 31, 2016) (stating that Enlist Duo can be used “[f]or suppression” of common milkweed).



It is well-documented that glyphosate has caused significant harm to monarch butterflies by destroying milkweed. The increased use of glyphosate across the country, spurred by widespread adoption of glyphosate-resistant GE crops (including Roundup Ready crops), has drastically reduced the presence of agricultural milkweed over the last decade. The pervasive suppression of milkweed has, in turn, contributed to a sharp decline in the monarch population. In 1997, before the widespread adoption of glyphosate-resistant crops, approximately one billion monarchs journeyed across the country between summer havens in the United States and Canada and a single winter home in Mexico.<sup>40</sup> In 2014, only about 33 million monarchs completed that journey.<sup>41</sup> Experts—including at the Department of Agriculture—have warned that the annual monarch migration may be in danger of effectively vanishing.<sup>42</sup>

In its proposed registration for Enlist Duo, EPA concluded that “no new assessment is needed for glyphosate” because “no new use patterns and no new exposures for glyphosate are being considered with this registration action.”<sup>43</sup> However, EPA has *never* considered adverse

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<sup>40</sup> Michael Wines, *Migration of Monarch Butterflies Shrinks Again Under Inhospitable Conditions*, N.Y. Times, Jan. 29, 2014; Sylvia Fallon, *Monarch Butterfly Population Hits a New Low*, *Switchboard: Natural Resources Defense Council Staff Blog* (Jan. 29, 2014), [http://switchboard.nrdc.org/blogs/sfallon/monarch\\_butterfly\\_population\\_h.html](http://switchboard.nrdc.org/blogs/sfallon/monarch_butterfly_population_h.html).

<sup>41</sup> Monarch Watch, *supra* note 11, at fig.1.

<sup>42</sup> See, e.g., L.P. Brower et al., *Decline of Monarch Butterflies Overwintering in Mexico: Is the Migratory Phenomenon at Risk?*, Insect Conservation and Diversity (2011); Natural Resources Conservation Serv., USDA, et al., *Pollinator Plants of the Central United States: Native Milkweeds 4* (2013) (“Unfortunately, the future of the monarch migration is at risk.”); Brice X. Semmens, *supra* note 11; see also Wines, *supra* note 40; Michael Wines, *Monarch Migration Plunges to Lowest Level in Decades*, N.Y. Times, Mar. 13, 2013; see also Tracy Wilkinson, *U.S., Mexico and Canada are Asked to Protect Monarch Butterflies*, L.A. Times, Feb. 14, 2014.

<sup>43</sup> EPA, Proposed Registration Decision, at 2.

impacts to monarchs in conjunction with any previous registration of a glyphosate-containing pesticide. Accordingly, the agency must consider those impacts now.

Like glyphosate, 2,4-D also poses a risk to monarchs. 2,4-D is an active ingredient in herbicides “registered to control broadleaf weeds,”<sup>44</sup> and milkweed has been classified as a broadleaf plant.<sup>45</sup> Accordingly, EPA must also consider the risks that 2,4-D poses to monarchs through its destruction of milkweed.

### **1. Glyphosate use has increased significantly since reregistration in 1993**

EPA first registered a glyphosate-containing pesticide product in 1974.<sup>46</sup> As a non-selective herbicidal active ingredient,<sup>47</sup> glyphosate does not discriminate between target and non-target plants. Because of its harm to crops, glyphosate’s early use was relatively limited.<sup>48</sup> In a typical year between 1989 and 1991, 18.7 million pounds of glyphosate were applied as an active ingredient to between 13 and 20 million acres.<sup>49</sup> Out of this aggregate amount of glyphosate used across all types of acreage, 1.1 to 1.2 million pounds of the chemical were applied to between 1.3 and 1.7 million acres of corn, 2.2 to 2.4 million pounds of the chemical were applied to between 2.6 and 4.8 million acres of soybeans, and 225-375 thousand pounds of the chemical were

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<sup>44</sup> *Id.* at 1.

<sup>45</sup> Coll. of Agric. Scis., Penn State, Broadleaf Weeds, <http://plantscience.psu.edu/research/centers/turf/extension/plant-id/broadleaf> (last visited Nov. 28, 2016).

<sup>46</sup> Nat’l Pesticide Info. Ctr., Glyphosate Technical Fact Sheet 1, *available at* <http://npic.orst.edu/factsheets/glyphotech.pdf>.

<sup>47</sup> 2009 Glyphosate Final Work Plan, *supra* note 3, at 2.

<sup>48</sup> See J.M. Pleasants & K.S. Oberhauser, *Milkweed Loss in Agricultural Fields Because of Herbicide Use: Effects on the Monarch Butterfly Population*, Insect Conservation and Diversity 1, 2 (2012).

<sup>49</sup> 1993 Glyphosate R.E.D., *supra* note 8, at 9.

applied to between 300 thousand to 1 million acres of cotton.<sup>50</sup> In deciding to reregister glyphosate in 1993, EPA assumed that the pesticide was used in accordance with these estimates.<sup>51</sup>

Following the reregistration of glyphosate in 1993, however, genetically-modified, glyphosate-resistant crops were introduced in American agriculture. Glyphosate-resistant soybeans first appeared in 1996,<sup>52</sup> followed by glyphosate-resistant corn in 1997,<sup>53</sup> and glyphosate-resistant corn in 1998.<sup>54</sup> By 1999, glyphosate-resistant soybeans comprised the majority of all soybean crops.<sup>55</sup> The ascendancy of glyphosate-resistant crops is reflected in data from the corn-soy belt; by 2006, for example, 75% of farmers in Iowa reported planting continuous glyphosate-resistant crops.<sup>56</sup> By 2016, 94% of soybean crops were glyphosate-resistant, as well as 89% of both corn and cotton crops.<sup>57</sup>

The proliferation of glyphosate-resistant crops facilitated a dramatic expansion in glyphosate use.<sup>58</sup> In reregistering glyphosate-based herbicides in 1993, EPA estimated that 2.2-

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<sup>50</sup> *Id.* at 7-8.

<sup>51</sup> *See id.* at 8-9.

<sup>52</sup> G.M. Dill, et al., *Glyphosate-resistant Crops: Adoption, Use and Future Considerations*, 64 Pest Mgmt. Sci. 326, 326 (2008).

<sup>53</sup> Ginger G. Light, *Yield of Glyphosate-Tolerant Cotton as Affected by Topical Glyphosate Applications on the Texas High Plains and Rolling Plains*, J. of Cotton Sci. 7:231-235 (2003).

<sup>54</sup> Pleasants & Oberhauser, *supra* note 48, at 2.

<sup>55</sup> R.G. Hartzler, *Reduction in Common Milkweed (Asclepias Syriaca) Occurrence in Iowa Cropland from 1999 to 2009*, 29 Crop Protection 1542, 1542 (2010).

<sup>56</sup> *Id.*

<sup>57</sup> *See* Econ. Research Serv., *supra* note 33.

<sup>58</sup> *See* Pleasants & Oberhauser, *supra* note 48, at 1-2; Ctr. for Food Safety, Comments to EPA on Opening of Glyphosate Docket for Registration Review 2-8 (Sept. 21, 2009) [hereinafter CFS 2009 Comments].

2.4 million pounds of glyphosate were used on soybeans annually, 1.1-1.2 million pounds were used on corn annually, and 225-375 thousand pounds were used on cotton annually.<sup>59</sup>

Subsequently, in a 2008 screening level usage analysis based only on reported numbers, EPA estimated that, in an average year between 2004 and 2011, 95% of all soybean crops, 60% of all corn crops, and 85% of all cotton crops were treated with glyphosate; this required 86.4 million pounds of glyphosate for soybeans annually, 54.6 million pounds of glyphosate for corn annually, and 17.5 million pounds of glyphosate for cotton annually.<sup>60</sup> Right before the adoption of glyphosate-resistant crops, in 1995, approximately 7.6 million pounds of glyphosate were used on soybeans, 2.6 million pounds were used on corn, and 1 million pounds were used on cotton. After the widespread adoption of glyphosate-resistant crops, in 2014, approximately 122.5 million pounds of glyphosate were used on soybeans, 68.9 million pounds were used on corn, and 17.4 million pounds were used on cotton.<sup>61</sup>

A recent analysis suggests that agricultural use of glyphosate active ingredient reached approximately 250 million pounds in 2014—compared to 800,000 pounds in 1974, when the first glyphosate-based herbicide was registered.<sup>62</sup> In 1990, closely preceding EPA's reregistration of glyphosate in 1993, a total of 12.7 million pounds of glyphosate were used in the U.S., with agricultural uses comprising about 58% (7.4 million pounds).<sup>63</sup> Since then, agricultural uses of

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<sup>59</sup> 1993 Glyphosate R.E.D., *supra* note 8, at 7-8.

<sup>60</sup> Memorandum (EPA Updated Screening Level Usage Analysis (SLUA) Report in Support of Registration Review of Glyphosate) from Sepehr Haddad, Env'tl. Prot. Specialist, Bio. and Econ. Analysis Div., EPA, to Carissa Cyran, Chem. Review Manager, Pesticide Re-Evaluation Div., EPA (Dec. 6, 2012).

<sup>61</sup> *See Benbrook*, *supra* note 8, at 6 & tbl. 3.

<sup>62</sup> *Id.* at 5 tbl.1.

<sup>63</sup> *See id.*

glyphosate have skyrocketed, mainly due to the so-called “Roundup Ready” genetically engineered glyphosate-tolerant crops in the mid-1990s.<sup>64</sup> In 2014, the most recent year for which data are available, a total of 276 million pounds were used in the United States, with agricultural uses making up over 90% of that (250 million pounds).<sup>65</sup> In other words, use of glyphosate has increased approximately 10-fold following the adoption of glyphosate-resistant crops.

In 2009, EPA initiated a registration review for glyphosate.<sup>66</sup> Extensive public comment submitted to the agency identified “profound changes in the usage patterns” of glyphosate, driven in part by the “widespread adoption of transgenic, glyphosate-resistant” crops.<sup>67</sup> EPA affirmed its “aware[ness] of the tremendous growth in the use of glyphosate since it was reregistered, and its relationship with the development of herbicide tolerant crops.”<sup>68</sup> The agency moreover recognized that “[a]ccurate estimates of current use patterns will indeed be important for evaluating the . . . environmental effects of glyphosate.”<sup>69</sup> EPA’s registration review for glyphosate was scheduled for completion in 2015.<sup>70</sup> However, the agency has yet to release even a preliminary risk assessment for public comment.

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<sup>64</sup> *See id.* at 5 & tbl.1.

<sup>65</sup> *See id.*

<sup>66</sup> Registration Review; Glyphosate Docket Opened for Review and Comment, 74 Fed. Reg. 36,217 (July 22, 2009).

<sup>67</sup> CFS 2009 Comments, *supra* note 58, at 1, 3.

<sup>68</sup> Memorandum (BEAD Responses to Selected Glyphosate Comments) from Derek Berwald, Economist, Bio. and Econ. Analysis Div., EPA, to Jude Andreasen, Chemical Review Manager, Pesticide Re-Evaluation Div., EPA 5 (Dec. 16, 2009).

<sup>69</sup> *Id.*

<sup>70</sup> 2009 Glyphosate Final Work Plan, *supra* note 3, at 4.

**2. Increased use of glyphosate has contributed significantly to monarch population decline**

The expanded use of glyphosate has contributed to a sharp decrease in monarch population levels through the herbicide's large-scale suppression of milkweed.<sup>71</sup> Milkweed is a perennial plant in the *Asclepiadaceae* family, and common milkweed is native to North-Central and Northeastern United States.<sup>72</sup> Members of this plant family constitute the sole food source for monarch larvae.<sup>73</sup> Stable isotope analysis has revealed that 50% of the North American monarch population that overwinters in Mexico fed on milkweed in the Midwestern United States during their lifecycle.<sup>74</sup>

Glyphosate is applied in part to control milkweed.<sup>75</sup> Because glyphosate is also detrimental to crops, however, its use was not widespread until the creation and approval of glyphosate-resistant crops.<sup>76</sup> The rapid replacement of traditional crop strains with glyphosate-resistant strains substantially accelerated an increase in the use of glyphosate, contributing to a significant decline in milkweed communities.<sup>77</sup> Herbicides are used more frequently, and at

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<sup>71</sup> See generally J.M. Pleasants, *Monarch Butterflies and Agriculture*, in *Monarchs in a Changing World: Biology and Conservation of an Iconic Insect* 169 (Karen Oberhauser, Sonia Alitzer, & Kelly Nail eds., 2015).

<sup>72</sup> R.G. Hartzler & D.D. Buhler, Occurrence of Common Milkweed (*Asclepias syriaca*) in Cropland and Adjacent Areas, 19 Crop Protection 363, 363 (2000).

<sup>73</sup> *Id.*

<sup>74</sup> Wassenaar, *supra*, note 10, at 15439.

<sup>75</sup> See Pleasants & Oberhauser, *supra* note 48, at 2; W.A. Plaine et al., *Weed and Herbicide-resistant Soybean (Glycine max) Response to Glufosinate and Glyphosate Plus Ammonium Sulfate and Pelargonic Acid*, 14 Weed Tech. 667, 667 (2000).

<sup>76</sup> See *supra* Section III.A.

<sup>77</sup> See *id.*

higher rates, when applied to herbicide-resistant crops.<sup>78</sup> This is particularly destructive to milkweed, because herbicides that cause limited damage to weeds when applied at lower rates are often much more damaging when sprayed at higher rates.<sup>79</sup> In addition, milkweed tends to regrow when it is mowed, damaged by tilling, or treated with herbicides that are applied before milkweed shoots emerge in late spring.<sup>80</sup> But when herbicides are paired with herbicide-resistant crops, they can be applied later in the growing season during the milkweed plant's most vulnerable flowering stage.<sup>81</sup>

The relationship between use of herbicides on herbicide-resistant crops and declining milkweed is strikingly exemplified by data concerning glyphosate. Prior to the widespread adoption of glyphosate-resistant crops, for example, a 1999 survey of croplands in Iowa found that approximately 50% of all corn and soybean fields contained common milkweed.<sup>82</sup> By 2009, milkweed was found in only 8% of surveyed fields.<sup>83</sup> Additionally, the area occupied by common milkweed in these fields was reduced by 90%.<sup>84</sup> Since 1996, the adoption of herbicide-resistant corn and soybeans has contributed to approximately 150 million acres of habitat loss for monarchs; this loss is likely to increase as uncultivated lands are increasingly converted into

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<sup>78</sup> CFS 2014 Comments, *supra* note 19, at 13-14.

<sup>79</sup> *Id.* at 14. Indeed, the proposed label for Enlist Duo, which characterizes milkweed as a "perennial weed," says "Perennial weeds may require higher rates [of herbicide application] for best control." EPA, *supra* note 39, at 19-20.

<sup>80</sup> *Id.* at 13.

<sup>81</sup> *Id.*

<sup>82</sup> Hartzler, *supra* note 55, at 1542.

<sup>83</sup> *Id.*

<sup>84</sup> *Id.*



cropland planted with glyphosate-resistant crops.<sup>85</sup>

There has been a pronounced loss of both agricultural and non-agricultural habitat for monarchs since the adoption of glyphosate-resistant crops.<sup>86</sup> Agricultural milkweed has disappeared at a faster rate, however, and its loss is particularly detrimental to monarchs.<sup>87</sup> Studies have shown that monarchs in the Midwest preferentially use milkweed in agricultural habitat versus non-agricultural habitat, with soy and corn fields producing over 70 times more monarchs than non-agricultural habitats in Iowa, Minnesota, and Wisconsin.<sup>88</sup> This pattern of monarchs preferring agricultural over non-agricultural sites was confirmed by Pleasants and Oberhauser in 2012.<sup>89</sup>

The disappearance of milkweed along monarch migratory paths has had a significant impact on monarch production.<sup>90</sup> Each spring, the monarch population embarks on a multi-generational migration that begins in the forests of central Mexico.<sup>91</sup> The butterflies fly north

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<sup>85</sup> See Chip Taylor, *Monarch Population Status*, MonarchWatch.org (Jan. 29, 2014, 12:10 PM), <http://monarchwatch.org/blog/2014/01/monarch-population-status-20/>; see also Scott Faber et al., *Plowed Under: How Crop Subsidies Contribute to Massive Habitat Losses* 8 (2012), available at [http://static.ewg.org/pdf/plowed\\_under.pdf](http://static.ewg.org/pdf/plowed_under.pdf) (documenting numbers of acres of grasslands, wetlands, and shrub lands converted to corn and soybean farmland between 2008 and 2011).

<sup>86</sup> See Pleasants & Oberhauser, *supra* note 48, at 3-5.

<sup>87</sup> See *id.* at 3-6

<sup>88</sup> See K.S. Oberhauser et al., *Temporal and Spatial Overlap Between Monarch Larvae and Corn Pollen*, 98 Proc. Nat'l Acad. Sci. U.S. 11913, 11917 (2001).

<sup>89</sup> Pleasants & Oberhauser, *supra* note 48, at 8.

<sup>90</sup> See *id.* at 1-10; see also CFS, *supra* note 10.

<sup>91</sup> U.S. Forest Serv., USDA, Monarch Butterfly Migration and Overwintering, [http://fs.fed.us/wildflowers/pollinators/Monarch\\_Butterfly/migration/index.shtml](http://fs.fed.us/wildflowers/pollinators/Monarch_Butterfly/migration/index.shtml) (last visited Nov. 29, 2016); see also Stephen B. Malcolm et al., *Spring Recolonization of Eastern North American by the Monarch Butterfly: Successive Brood or Single Sweep Migration?*, in *Biology and Conservation of the Monarch Butterfly* 253 (SB Malcolm & MP Zalucki eds. 1993).

across the United States, reproducing along the way.<sup>92</sup> By mid-to-late summer, over the span of four to five generations, the population covers much of the United States reaching to parts of southern Canada.<sup>93</sup> Because the migration occurs over multiple generations and milkweed is the only plant the monarch caterpillars can feed on, the monarch butterflies need to find milkweed across their entire migratory pathway.<sup>94</sup> In the fall, the last generation of monarchs flies back to the same forests in Mexico where the population's journey began.<sup>95</sup> The butterflies overwinter in Mexico until spring, when the migration cycle begins again.<sup>96</sup> The entire migration can span up to 3,000 miles.<sup>97</sup>

The monarch population cannot complete this extraordinary migration unless it encounters sufficient milkweed along the migratory pathway. Because monarch caterpillars depend solely on milkweed plants for their development, migrating female monarch butterflies seek out milkweed on which to lay their eggs.<sup>98</sup> As milkweed disappears from the monarch migratory path, adult females must now travel further and expend more energy to find milkweed plants on which to lay their eggs.<sup>99</sup> With depleted body fat, as well as fewer milkweed host plants on which to deposit eggs, the butterflies lay fewer eggs and face a heightened risk of dying

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<sup>92</sup> *Id.*

<sup>93</sup> *Id.*

<sup>94</sup> *See id.*; Hartzler, *supra* note 41, at 363.

<sup>95</sup> *See* U.S. Forest Serv., *supra* note 91.

<sup>96</sup> *Id.*

<sup>97</sup> *Id.*

<sup>98</sup> *See* D.T. Tyler Flockhart et al., *Unravelling the Annual Cycle in a Migratory Animal: Breeding-Season Habitat Loss Drives Population Declines of Monarch Butterflies* 7, *J. Animal Ecology* (2014); Malcolm, *supra* note 91, at 253.

<sup>99</sup> Wines, *supra* note 40.

before having the chance to reproduce.<sup>100</sup> With fewer eggs laid, the number of next-generation monarchs available to complete the migration and return to Mexico diminishes.<sup>101</sup> Reduction of milkweed also decreases the number of caterpillars that survive to adulthood, by intensifying competition over a limited food supply.<sup>102</sup>

Over the period marked by increased glyphosate-use and planting of glyphosate-resistant corn and soy, Pleasants and Oberhauser examined monarch production in the Midwest as measured by the number of eggs and larvae on milkweed.<sup>103</sup> They found a 58% decline in milkweed across the Midwest landscape and an 81% decrease in monarch production in the Midwest from 1999 to 2010.<sup>104</sup> During this time, there was also a 65% decrease in the size of the entire monarch population overwintering in Mexico.<sup>105</sup> By 2014, the population dropped another 22%.<sup>106</sup>

Monarch experts have found that the most significant driving force behind the butterfly's decline is the loss of milkweed in the United States due to the widespread use of herbicides, particularly those containing glyphosate, on herbicide-resistant crops.<sup>107</sup> A 2014 study examining

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<sup>100</sup> *Id.*; see Flockhart, *supra* note 98, at 7-8.

<sup>101</sup> See Pleasants & Oberhauser, *supra* note 48, at 5.

<sup>102</sup> See Flockhart, *supra* note 98, at 2, 7-8.

<sup>103</sup> See Pleasants & Oberhauser, *supra* note 48, at 1.

<sup>104</sup> See *id.*

<sup>105</sup> *Id.* at 8; see also Brower, *supra* note 42, at 1 (2011).

<sup>106</sup> See Monarch Watch, *supra* note 11, at fig.1.

<sup>107</sup> See, e.g., Brower, *supra* note 23, at D.T. Tyler Flockhart et al., *Unravelling the Annual Cycle in a Migratory Animal: Breeding-Season Habitat Loss Drives Population Declines of Monarch Butterflies*, J. Animal Ecology, at 7-8 (2014); Pleasants, *supra* note 71; Pleasants & Oberhauser, *supra* note 48; see also See Reply Decl. of Dr. Sylvia Fallon in Supp. of Pet'r

the various threats to the monarch population confirmed that “[r]ecent population declines stem from reduction in milkweed host plants in the United States that arise from increasing adoption of genetically modified crops and land-use change, not from climate change or degradation of forest habitats in Mexico.”<sup>108</sup> The study concluded that “conserving monarch butterflies by addressing the negative impacts of changing land-use and the adoption of genetically-modified, herbicide resistant crops on host plant abundance is the highest conservation priority,” and that “reducing the negative effects of host plant loss on the breeding groups is the top conservation priority to slow or halt future population declines of monarch butterflies in North America.”<sup>109</sup> As the Natural Resources Conservation Service of the USDA has likewise recognized, the “documented declines in milkweed habitat and monarch breeding potential illustrate the urgent need to protect existing milkweed populations.”<sup>110</sup>

Notably, because monarch butterflies produce 3.9 times more offspring on milkweed within agricultural fields than outside of them, a new analysis estimates that while 850 million agricultural milkweed plants have been eliminated since 1999, it will require a minimum of 1.6 billion non-agricultural milkweed plants to significantly reduce the monarch butterfly population’s extinction probability.<sup>111</sup>

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NRDC’s Mot. for Stay Pending Review, *NRDC v. EPA*, No. 14-73353, ECF No. 36-2, at 10-11 (9th Cir. Feb. 17, 2015).

<sup>108</sup> Flockhart, *supra* note 107, at 1 (2014).

<sup>109</sup> *Id.* at 1, 8.

<sup>110</sup> Natural Resources Conservation Serv., USDA, *supra* note 42, at 7 (2013).

<sup>111</sup> John Pleasants, *Milkweed Restoration in the Midwest of Monarch Butterfly Recovery: Estimates of Milkweeds Lost, Milkweeds Remaining and Milkweeds that Must Be Added to Increase the Monarch Population*, Insect Conservation and Diversity (2016).

A series of studies in 2015-2016 questioned whether the decline in monarch butterfly numbers was due to the loss of milkweed and postulated that the decline may instead be due to the loss of nectar sources during the fall migration. However, subsequent studies identified a number of flaws with those analyses and once again supported the conclusion that the loss of milkweed is the primary driver of the monarch decline.<sup>112</sup>

According to a survey taken in 2014, the area inhabited by monarchs overwintering in Mexico shrunk to an all-time low: a mere 0.67 hectares, the equivalent of about one-and-a-quarter football fields.<sup>113</sup> Not only was this a record low, but it was only 56% percent of the previous year's acreage, which was itself a record low.<sup>114</sup> The area of winter habitat occupied by monarchs, which has been surveyed annually since 1993, provides a proxy for the number of butterflies that survive the arduous, 2,500-plus-mile journey between Canada and Mexico.<sup>115</sup> The 2014 winter survey reflected a remaining population of only about 33.5 million butterflies—down from a long-term average annual count of approximately 350 million individuals over the last 15 years.<sup>116</sup> Although overwintering monarchs occupied a slightly increased area of

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<sup>112</sup> John M. Pleasants et al., *Conclusion of No Decline in Summer Monarch Population Not Supported*, *Annals of the Entomological Society of Am.* (Feb. 4, 2016); Chip Taylor et al., *Is the Monarch Decline Due to an Increase in Mortality During the Fall Migration?* (Nov. 2, 2016), available at <http://monarchwatch.org/blog/2016/11/02/tagging-results-and-the-monarch-decline/>.

<sup>113</sup> Wines, *supra* note 40; see E. Rendón-Salinas & G. Tavera-Alosno, *Monitoreo de la Superficie Forestal Ocupada por las Colonias de Hibernación de la Mariposa Monarca en Diciembre de 2013*, at 1, [http://awsassets.panda.org/downloads/monitoreo\\_mariposa\\_monarca\\_en\\_mexico\\_2013\\_2014.pdf](http://awsassets.panda.org/downloads/monitoreo_mariposa_monarca_en_mexico_2013_2014.pdf) (last visited June 30, 2014); World Wildlife Fed'n, *La Migración de la Mariposa Monarca en Riesgo de Desaparecer*, WWF Mexico (June 30, 2014), <http://www.wwf.org.mx/?214870/La-migracin-de-la-mariposa-Monarca-en-riesgo-de-desaparecer>.

<sup>114</sup> Wines, *supra* note 40.

<sup>115</sup> *Id.*

<sup>116</sup> Fallon, *supra* note 40.

approximately 4.1 hectares in 2016, a severe winter storm killed millions of monarchs before they were able to begin their northward migration,<sup>117</sup> and estimates from their southward migration this year suggest the overwintering population will occupy a mere 1.13 hectares.<sup>118</sup>

The migrating monarch population has so diminished that its prospects for recovering are fading.<sup>119</sup> Continued milkweed loss renders the population susceptible to further decline, compromising its ability to withstand additional stressors such as severe weather, freezing temperatures, disease, predation, and deforestation.<sup>120</sup> In 2002, for example, a single storm killed approximately 500 million monarchs (approximately 75% of the overwintering population)—that is, over three times the 150 million monarchs overwintering in Mexico during the 2015-16 winter.<sup>121</sup> Severe storms are not uncommon. In 2004, for example, another serious storm killed

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<sup>117</sup> See Associated Press, *Millions of Monarch Butterflies Killed off by Mexico Storms* (Aug. 24, 2016), available at <https://weather.com/science/nature/news/mexico-monarch-butterflies-death-winter-weather>; see Associated Press, *Experts: Mexico Storms Led to Deaths of Millions of Monarchs* (Aug. 23, 2016), available at <http://www.whio.com/news/world/experts-mexico-storms-led-deaths-millions-monarchs/EAAZTTq18idOIMXmDt2M/>.

<sup>118</sup> Monarch Watch, Monarch Population Status (Sept. 14, 2016), <http://monarchwatch.org/blog/2016/09/14/monarch-population-status-29/>.

<sup>119</sup> Wines, *supra* note 40.

<sup>120</sup> Brower, *supra* note 42, at 3-4; Pleasants & Oberhauser, *supra* note 48, at 9; Wines, *supra* note 40.

<sup>121</sup> Monarch Watch, *Why We Still Tag Monarchs*, at 1 (Sept. 15, 2016), available at <http://monarchwatch.org/tagmig/2016-datasheet-mini.pdf>; compare Brower, *supra* note 11, at 162, with Monarch Watch, *supra* note 11 (reporting that overwintering monarchs occupied 4.01 hectares during the 2015-2016 winter season), and World Wildlife Fund, *Aumenta la Superficie Ocupada por la Mariposa Monarca en Los Santuarios Mexicanos* (Feb. 26, 2010), <http://www.wwf.org.mx/?262370/Aumenta-superficie-ocupada-por-mariposa-monarca-en-santuarios-mexicanos> (same); See also Semmens, *supra* note 11, at 5-6 (explaining that “tightly clustered overwintering colonies convey important microclimate advantages that diminish as colony size decreases,” so that “[d]iminishing colony size can therefore result in higher winter mortality rates”).



about 70% of the overwintering monarch population.<sup>122</sup> Subsequently, in 2010, a single storm killed approximately fifty percent of the monarchs overwintering in Mexico—that is, more than the total number of overwintering monarchs measured four years later in 2014.<sup>123</sup>

Given its current size, the monarch population is susceptible to complete eradication by comparable storms.<sup>124</sup> The smaller the population becomes, the more vulnerable it is to these kinds of random events.<sup>125</sup> Additional destruction of milkweed habitat in the butterflies' breeding ground thus puts the monarch at further risk. The population is so precariously small that experts—including those at the Department of Agriculture—have warned that the monarch migration may be coming to an end.<sup>126</sup> In fact, a study published in 2016 found a “high probability of quasi-extinction over the next two decades.”<sup>127</sup> The potential approval of new herbicide-resistant crops,<sup>128</sup> which may facilitate substantial increased use of other herbicides that further eliminate milkweed, poses an additional threat to monarchs. In the face of steep,

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<sup>122</sup> Monarch Watch, *supra* note 121.

<sup>123</sup> See Brower, *supra* note 42, at 4; *compare id.* at 2 (noting that overwintering monarch colonies occupied a total of 1.92 hectares during the 2009-2010 winter season), *with* Monarch Watch, *supra* note 11 (monarchs occupied a total of only 0.67 hectares, or 1.65 acres, during the winter of 2013-14).

<sup>124</sup> See Decl. of Dr. Sylvia Fallon in Supp. of Pet'r NRDC's Mot. for Stay Pending Review, *NRDC v. EPA*, No. 14-73353, ECF No. 15-2, at 10-11 (9th Cir. Dec. 18, 2014).

<sup>125</sup> *Id.* at 11.

<sup>126</sup> See *supra* note 42.

<sup>127</sup> See Semmens, *supra* note 11.

<sup>128</sup> See Animal Plant and Health Inspection Serv. (APHIS), USDA, *Petitions for Determination of Nonregulated Status*, [http://www.aphis.usda.gov/biotechnology/petitions\\_table\\_pending.shtml#not\\_reg](http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml#not_reg) (last visited Nov. 30, 2016) (listing genetically modified crops, including those with tolerance to various herbicides, for which petitions for determination of nonregulated status have been filed).

continuing population decline, the phenomenon of monarch migration is at risk of disappearing.<sup>129</sup>

**3. Increased use of 2,4-D also threatens to accelerate monarch population decline**

The 2,4-D component of Enlist Duo places monarchs at further risk. Like glyphosate, 2,4-D harms milkweed and thus monarchs. 2,4-D is an active ingredient in herbicides “registered to control broadleaf weeds,”<sup>130</sup> and milkweed has been classified as a broadleaf plant.<sup>131</sup> Particularly in light of the significant expanded use of 2,4-D that would result from registration of Enlist Duo, EPA must also consider the risks that 2,4-D poses to monarchs through its destruction of milkweed; it is not sufficient for EPA to examine whether 2,4-D is toxic to monarchs or other invertebrates.

**C. Enlist Duo May Pose Serious Risks to Human Health, Including Cancer Risks**

**1. EPA ignored current science on glyphosate’s human health risks**

Glyphosate is the most commonly used herbicide in the United States.<sup>132</sup> As discussed, use of glyphosate has increased over ten-fold since the years preceding its reregistration.<sup>133</sup> In the twenty-three years since EPA reregistered glyphosate, considerable new research has provided a greatly increased basis for concern about this chemical’s health impacts. Over 3,000 studies have been published in peer-reviewed journals, with some evidence suggesting glyphosate and

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<sup>129</sup> Natural Resources Conservation Serv., USDA, *supra* note 42, at 4 (“Unfortunately, the future of the monarch migration is at risk.”); Wines, *supra* note 40.

<sup>130</sup> EPA, Proposed Registration Decision, *supra*, at 1.

<sup>131</sup> Coll. of Agric. Scis., *supra* note 45.

<sup>132</sup> Benbrook, *supra*, note 8, at 1.

<sup>133</sup> *See supra* note 9 & accompanying text; *see supra* Section III.A.1.

glyphosate-based products may increase the risk of cancer, kidney toxicity,<sup>134</sup> pre-term deliveries, miscarriages, attention deficit hyperactivity disorder, neural tube defects, and birth defects.<sup>135</sup>

With respect to glyphosate's carcinogenicity, in 1985, a committee within EPA's Toxicology Branch concluded that glyphosate was a Category C oncogen,<sup>136</sup> meaning that there was evidence in animal studies that the chemical was possibly carcinogenic to humans.<sup>137</sup> In 1991, based in part on the diagnosis of a single additional tumor in a control group mouse,<sup>138</sup> EPA down-graded the classification of glyphosate as a Group E oncogen, meaning that there was "evidence of non-carcinogenicity for humans."<sup>139</sup> A peer review committee "emphasized, however, that designation of an agent in Group E is based on the available evidence at the time of evaluation and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances."<sup>140</sup>

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<sup>134</sup> Jayasumana C, Gunatilake S, Senanayake P. Glyphosate, Hard Water and Nephrotoxic Metals: Are They the Culprits Behind the Epidemic of Chronic Kidney Disease of Unknown Etiology in Sri Lanka? *Int J Environ Res Public Health*. 2014;11(2):2125-2147. doi:10.3390/ijerph110202125.

<sup>135</sup> Antoniou M, Habib MEM, Howard CV, et al. Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence. *J Environ Anal Toxicol*. 2012, S4:006. doi:10.4172/2161-0525.S4-006; and Garry VF, Schreinemachers D, Harkins ME, Griffith J. Pesticide applicators, biocides, and birth defects in rural Minnesota. *Environ Health Perspect*. 1996;104(4):394-399. doi:10.2307/3432683.

<sup>136</sup> William Dykstra, EPA, Memorandum re SECOND Peer Review of Glyphosate, at 3 (Oct. 30, 1991); *see also* William Dykstra, EPA, Memorandum re Glyphosate (Apr. 3, 1985).

<sup>137</sup> *See* EPA, Risk Assessment for Carcinogens, <http://www2.epa.gov/fera/risk-assessment-carcinogens> (Oct. 2, 2015).

<sup>138</sup> William Dykstra, EPA, Memorandum re Glyphosate, at 1-2 (Mar. 12, 1986).

<sup>139</sup> 1993 Glyphosate R.E.D., *supra* note 8, at 14; Dykstra (1991), *supra* note 136, at 1.

<sup>140</sup> Dykstra (1991), *supra* note 136, at 1.

In its decision to re-register glyphosate-containing pesticides in 1993, EPA relied on its 1991 designation of glyphosate as a Group E oncogen to conclude that glyphosate has low toxicity to humans.<sup>141</sup> There is no evidence in the administrative record that EPA reviewed any more recent studies on glyphosate's cancer risk before registering Enlist Duo. On the contrary, EPA states that "no new assessment is needed for glyphosate."<sup>142</sup>

The state of the science on glyphosate's carcinogenicity has, however, changed significantly since the early 1990s. In early 2015, IARC, the cancer evaluation branch of the World Health Organization, convened a meeting of 17 scientific experts from 11 countries to review the cancer data regarding glyphosate, and published their conclusions in a Monograph in mid-2016.<sup>143</sup> IARC is specifically qualified to conduct such chemical cancer assessments like this one. IARC has been conducting such reviews since 1971, and has evaluated over 900 agents, of which over 400 have been classified as "known," "probably," or "possibly" carcinogenic to humans.<sup>144</sup> The IARC Monographs are used and referenced world-wide by governments,

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<sup>141</sup> Dykstra (1991), *supra* note 136, at 13-14.

<sup>142</sup> EPA, Proposed Registration Decision, at 2.

<sup>143</sup> IARC, IARC Monographs Vol. 112 – Glyphosate (2015), *available at* <http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-09.pdf> [hereinafter IARC Glyphosate Monograph]; *see also* Kathryn Z. Guyton et al., IARC, *Carcinogenicity of Tetrachlorvinphos, Parathion, Malathion, Diazinon, and Glyphosate*, 16 *The Lancet Oncology* 490 (online ed. Mar. 2015; print ed. May 2015), *available at* <http://www.thelancet.com/pdfs/journals/lanonc/PIIS1470-2045%2815%2970134-8.pdf>; Press Release, IARC, World Health Org., IARC Monographs Volume 112: Evaluation of Five Organophosphate Insecticides and Herbicides (Mar. 12, 2015), *available at* <http://www.iarc.fr/en/media-centre/iarcnews/pdf/MonographVolume112.pdf>; IARC, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 112 (2015), <http://monographs.iarc.fr/ENG/Monographs/vol112/index.php> (last visited Nov. 23, 2016); CFS, *supra* note 12.

<sup>144</sup> International Agency for Research on Cancer, Monographs on the Evaluation of Carcinogenic Risks to Humans. Home webpage, *available at* <http://monographs.iarc.fr/>; *see also* Pearce N, Blair A, Vineis P, Ahrens W, Andersen A, Anto JM, Armstrong BK, Baccarelli AA,

organizations, scientists, and the public, including, in the United States, the NIH, OSHA, FDA, EPA, and state agencies.<sup>145</sup> According to the President’s Cancer Panel, IARC’s reports are the “gold standard” in evaluating evidence on cancer causation.<sup>146</sup>

The IARC experts unanimously voted to classify glyphosate as a probable (Group 2A) human carcinogen, based on three lines of evidence: “sufficient” evidence of cancer in mice and rats that were fed glyphosate over a several years; “strong” evidence of a cellular mechanism for cancer (genotoxicity and oxidative stress); and “limited” evidence from epidemiologic studies (non-Hodgkin lymphoma).<sup>147</sup> Almost 100 scientists from 25 countries published a commentary

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Beland FA, Berrington A, Bertazzi PA, Birnbaum LS, Brownson RC, Bucher JR, Cantor KP, Cardis E, Cherrie JW, Christiani DC, Cocco P, Coggon D, Comba P, Demers PA, Dement JM, Douwes J, Eisen EA, Engel LS, Fenske RA, Fleming LE, Fletcher T, Fontham E, Forastiere F, Frentzel-Beyme R, Fritschi L, Gerin M, Goldberg M, Grandjean P, Grimsrud TK, Gustavsson P, Haines A, Hartge P, Hansen J, Hauptmann M, Heederik D, Hemminki K, Hemon D, Hertz-Picciotto I, Hoppin JA, Huff J, Jarvholm B, Kang D, Karagas MR, Kjaerheim K, Kjuus H, Kogevinas M, Kriebel D, Kristensen P, Kromhout H, Laden F, LeBailly P, LeMasters G, Lubin JH, Lynch CF, Lynge E, 't Mannetje A, McMichael AJ, McLaughlin JR, Marrett L, Martuzzi M, Merchant JA, Merler E, Merletti F, Miller A, Mirer FE, Monson R, Nordby KC, Olshan AF, Parent ME, Perera FP, Perry MJ, Pesatori AC, Pirastu R, Porta M, Pukkala E, Rice C, Richardson DB, Ritter L, Ritz B, Ronckers CM, Rushton L, Rusiecki JA, Rusyn I, Samet JM, Sandler DP, de Sanjose S, Schernhammer E, Costantini AS, Seixas N, Shy C, Siemiatycki J, Silverman DT, Simonato L, Smith AH, Smith MT, Spinelli JJ, Spitz MR, Stallones L, Stayner LT, Steenland K, Stenzel M, Stewart BW, Stewart PA, Symanski E, Terracini B, Tolbert PE, Vainio H, Vena J, Vermeulen R, Victora CG, Ward EM, Weinberg CR, Weisenburger D, Wesseling C, Weiderpass E, Zahm SH. IARC monographs: 40 years of evaluating carcinogenic hazards to humans. *Environ Health Perspect.* 2015 Jun;123(6):507-14.

<sup>145</sup> See for example OSHA Appendix F to Section 1910.1200 – Guidance for Hazard Classifications Re: Carcinogenicity, <https://www.osha.gov/dsg/hazcom/hazcom-appendix-f.html>.

<sup>146</sup> President’s Cancer Panel, U.S. Dep’t of Health & Human Servs., Reducing Environmental Cancer Risk: What We Can Do Now 13 (2010), available at [http://deainfo.nci.nih.gov/advisory/pcp/annualReports/pcp08-09rpt/PCP\\_Report\\_08-09\\_508.pdf](http://deainfo.nci.nih.gov/advisory/pcp/annualReports/pcp08-09rpt/PCP_Report_08-09_508.pdf).

<sup>147</sup> See Guyton, *supra* note 143, at 1-2; IARC Glyphosate Monograph, *supra* note 143.

supporting the IARC evaluation.<sup>148</sup> Even a Monsanto-sponsored review and meta-analysis of epidemiologic studies supported this evaluation, reporting a statistically significant risk of non-Hodgkin lymphoma (NHL) cancers when glyphosate-exposed individuals were compared with individuals never exposed to glyphosate.<sup>149</sup>

In addition, EPA failed to adequately consider health risks, aside from acute toxicity, arising from synergistic interactions between glyphosate and other ingredients in Enlist Duo. A report submitted under contract to USDA in 1997—twenty years ago—warned that surfactants added to glyphosate products make them much more toxic, and that very little toxicity information is available about the formulated products.<sup>150</sup> Recognizing the hazards of these chemicals, in July 2016, European Union member states voted to ban them from glyphosate-

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<sup>148</sup> Portier CJ, Armstrong BK, Baguley BC, Baur X, Belyaev I, Bellé R, Belpoggi F, Biggeri A, Bosland MC, Bruzzi P, Budnik LT, Bugge MD, Burns K, Calaf GM, Carpenter DO, Carpenter HM, López-Carrillo L, Clapp R, Cocco P, Consonni D, Comba P, Craft E, Dalvie MA, Davis D, Demers PA, De Roos AJ, DeWitt J, Forastiere F, Freedman JH, Fritschi L, Gaus C, Gohlke JM, Goldberg M, Greiser E, Hansen J, Hardell L, Hauptmann M, Huang W, Huff J, James MO, Jameson CW, Kortenkamp A, Kopp-Schneider A, Kromhout H, Larramendy ML, Landrigan PJ, Lash LH, Leszczynski D, Lynch CF, Magnani C, Mandrioli D, Martin FL, Merler E, Michelozzi P, Miligi L, Miller AB, Mirabelli D, Mirer FE, Naidoo S, Perry MJ, Petronio MG, Pirastu R, Portier RJ, Ramos KS, Robertson LW, Rodriguez T, Rössli M, Ross MK, Roy D, Rusyn I, Saldiva P, Sass J, Savolainen K, Scheepers PT, Sergi C, Silbergeld EK, Smith MT, Stewart BW, Sutton P, Tateo F, Terracini B, Thielmann HW, Thomas DB, Vainio H, Vena JE, Vineis P, Weiderpass E, Weisenburger DD, Woodruff TJ, Yorifuji T, Yu IJ, Zambon P, Zeeb H, Zhou SF. Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA). *J Epidemiol Community Health*. 2016 Aug;70(8):741-5. Available at: <http://jech.bmj.com/content/early/2016/03/03/jech-2015-207005.full>.

<sup>149</sup> Chang ET, Delzell E. Systematic review and meta-analysis of glyphosate exposure and risk of lymphohematopoietic cancers. *J Environ Sci Health B*. 2016;51(6):402-34.

<sup>150</sup> Diamond GL, Durkin PR. Effects of Surfactants on the Toxicity of Glyphosate, with Specific Reference to RODEO Report submitted to Leslie Rubin, COTR, Animal and Plant Health Inspection Service (APHIS). Biotechnology, Biologics and Environmental Protection, Environmental Analysis and Documentation, United States Department of Agriculture, February 6, 1997, available at <http://www.fs.fed.us/foresthealth/pesticide/pdfs/Surfactants.pdf>.



based products including Roundup, noting that they are more toxic than glyphosate in terms of acute, short term, reproductive, and developmental toxicity, and that there is some evidence of DNA damage in vitro at high doses which may lead to cancer.<sup>151</sup>

Given the prolific increase in publications discussing glyphosate's health risks, including cancer risk, since EPA last completed a human health risk assessment for glyphosate in 1993, the agency cannot register Enlist Duo without first completing an updated human health risk assessment for glyphosate prior to registering Enlist Duo, taking into account public comments.

## **2. EPA cannot rely on an outdated dietary risk assessment for glyphosate**

The most recent dietary risk assessment that EPA has completed for glyphosate appears to be in the 1993 Reregistration Eligibility Decision for glyphosate.<sup>152</sup> But that outdated dietary risk assessment is inadequate to capture the true extent of human health risk that the glyphosate component of Enlist Duo would pose through dietary exposure.

Risk is a combination of both hazard and exposure—that is, how dangerous something may be, and how much of it a person is exposed to. Exposure information from food is just becoming available, and recent food residue testing data is showing that both glyphosate and 2,4-D are likely to be widespread contaminants in many popular foods. Earlier in 2016, the U.S. FDA initiated its first-ever program to start testing for glyphosate and for 2,4-D residues in foods, following a highly critical report in 2014 from the US Government Accountability

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<sup>151</sup> EFSA explains the carcinogenicity assessment of glyphosate. European Food Safety Authority: Pesticide Unit. Nov 12, 2015, *available at* [http://www.efsa.europa.eu/sites/default/files/4302\\_glyphosate\\_complementary.pdf](http://www.efsa.europa.eu/sites/default/files/4302_glyphosate_complementary.pdf).

<sup>152</sup> 1993 Glyphosate R.E.D., *supra* note 8.

Office.<sup>153</sup> The limited results available thus far are concerning. For example, FDA analysis has already reported that some honey samples had glyphosate residues as high as 650 parts per billion (ppb) well over the limit of 50 ppb allowed in the European Union; in the US the EPA has no legal tolerance for glyphosate in honey at this time.<sup>154</sup> FDA has also reported that some infant oat cereal samples had glyphosate residues as high as 1,560 ppb.<sup>155</sup> FDA has not set a legal tolerance for glyphosate on oat either.<sup>156</sup> Without a robust set of food residue data for both glyphosate and 2,4-D, EPA cannot fully account for exposures through food for the population—including pregnant women and children—and therefore EPA cannot accurately calculate risk. A recent report from two public interest groups, Food Democracy Now! and the Detox Project, conducted testing and reported glyphosate residues in many commonly eaten foods including breakfast cereals and snack foods.<sup>157</sup> Particularly given the massive expansion in use of glyphosate since the time many tolerances were set, the dearth of updated exposure information, including food residue testing, represents a very significant data gap and possibly a serious health threat to much of the American population.

Rather than taking into consideration all the foods on which glyphosate residues appear, EPA's dietary assessment of glyphosate is based on only 85 tolerances—the allowable upper

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<sup>153</sup> GAO (2014). FDA and USDA Should Strengthen Pesticide Residue Monitoring Programs and Further Disclose Monitoring Limitations. U.S. Government Accountability Office. GAO-15-38, October 7, 2014, available at <http://www.gao.gov/products/GAO-15-38>.

<sup>154</sup> FDA, Method Development/Validation of the Direct Determination of Glyphosate, Glufosinate, and AMPA in Food by LC/MS, at 27 (2016), available at <http://www.nacrw.org/2016/presentations/O-27.pdf>.

<sup>155</sup> *Id.* at 30.

<sup>156</sup> See 40 C.F.R. § 180.364.

<sup>157</sup> Food Democracy Now! and the Detox Project, Glyphosate: Unsafe on Any Plate (Nov. 2016), available at <http://www.fooddemocracynow.org/campaign/breaking-alarming-levels-monsantos-glyphosate-found-americas-food>.

limits of pesticide residues—on foods.<sup>158</sup> EPA must consider the aggregate of all exposures to glyphosate, including all dietary (food and drinking water) and residential uses of the chemical. The aggregate of all these exposures must not exceed the reference dose (RfD), presuming daily exposures over a lifetime and including sensitive and vulnerable populations such as pregnant women and children, and populations with unusual eating patterns. The aggregate or total of all exposures from all sources is often called the ‘risk cup’ by EPA, and it must not “overflow,” or exceed the RfD.

Unfortunately, EPA has failed to include some foods that FDA has recently shown to be contaminated with glyphosate, such as honey and instant oatmeal; it is very likely that a more realistic aggregate dietary assessment would exceed the RfD, that is, the cup would overflow. This is particularly important because these are foods commonly eaten by young children. EPA determined in its 1993 RED that the subgroup most highly exposed is non-nursing infants under one year old,<sup>159</sup> making this group a target for possible unsafe exposures (exceeding the RfD) due to cereals (often corn-based) introduced into an infant’s diet about mid-way through the first year of life.

Because EPA includes an unrealistically limited set of foods in its “risk cup” for glyphosate, the tolerances for the 85 foods that EPA did include in that risk cup—including corn, soy, and cotton—are likely too high. Furthermore, based on new information about the health risks posed by glyphosate—including cancer risk—that has arisen since 1993, the “risk cup” for glyphosate should be much smaller. In other words, the RfD is too high and not protective of human health, particularly for vulnerable populations including children and infants. In fact, the

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<sup>158</sup> 1993 Glyphosate R.E.D. Facts, *supra* note 5, at 3.

<sup>159</sup> 1993 Glyphosate R.E.D., *supra* note 8, at 48.

1993 Glyphosate R.E.D. uses an excessively high RfD of 2 mg/kg/day, whereas EPA has now recognized the RfD to be 1.75 mg/kg/day.<sup>160</sup> Insofar as EPA is relying on the dietary risk assessment in the 1993 R.E.D. for glyphosate to assess Enlist Duo's human health risk, that reliance is thus flawed and leads EPA to underestimate the human health risk posed by glyphosate through dietary exposure. EPA must conduct a new dietary risk assessment for glyphosate, using a sufficiently protective RfD and taking into consideration current data about likely exposures to glyphosate through food.

### **3. EPA's human health risk assessment for 2,4-D suffers from serious flaws**

#### **i. EPA ignored current science on 2,4-D's cancer risk**

In 2015, IARC classified 2,4-D as a "possible" human carcinogen (Group 2B) based primarily on strong evidence in cellular studies that 2,4-D causes the type of cellular damage that can lead to cancer (oxidative stress and immunosuppression), as well as limited evidence of cancer in laboratory animal studies.<sup>161</sup> In proposing to register Enlist Duo, however, EPA ignored IARC's recent cancer finding for 2,4-D, reasoning that "2,4-D has been classified as a Category D chemical, i.e., not classifiable as to human carcinogenicity. A quantitative cancer risk assessment is not required."<sup>162</sup> But this classification was made twenty years ago, back in

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<sup>160</sup> Compare *id.* at 19, with EPA, 76 Fed. Reg. 19701, 19703 (Apr. 8, 2011); see also EPA, 78 Fed. Reg. 25396.

<sup>161</sup> See *supra* note 14.

<sup>162</sup> EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide Tolerant Cotton, at 5, 35 (2016), available at <https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0594-0009>; accord EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean, at 4, 37 (2013).

1996.<sup>163</sup> In light of IARC's recent cancer finding, and the post-1996 studies that contributed to this finding,<sup>164</sup> it is inappropriate for EPA to rely on its outdated carcinogenicity classification for 2,4-D in registering Enlist Duo.

**ii. EPA must set reference doses (RfDs) or population adjusted doses (PADs) for 2,4-D that are adequately protective of sensitive developmental endpoints (e.g, brain development) and of human health**

In order to be protective of infants, children, and pregnant women, EPA must use, at a minimum, a developmental Lowest Observed Adverse Effect Level (LOAEL) of 5 mg/kg/day with a 10X uncertainty factor as the point of departure for dietary exposure of sensitive populations. EPA itself has stated that “[i]f a LOAEL is used, another uncertainty factor, generally 10x, is also used.”<sup>165</sup> EPA's current point of departure, 25 mg/kg/day, is not adequately protective against thyroid toxicity and population health.

This additional 10x uncertainty factor applied to the LOAEL is *separate from, and in addition to*, the additional 10x children's uncertainty factor discussed below that is presumptively required to ensure that pesticide tolerances are protective of children under the Food Quality Protection Act (FQPA), 21 U.S.C. § 346a(b)(2)(C)(ii). An adequately protective Population Adjusted Dose of 0.0005 mg/kg/day [with a 10X LOAEL to NOAEL conversion, a 10X uncertainty factor for animal to human extrapolation (interspecies), 10X difference between

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<sup>163</sup> EPA, Reregistration Eligibility Decision for 2,4-D, at 19 (2005) [hereinafter 2005 2,4-D R.E.D.] (“2,4-D has been classified as a Category D chemical (i.e., not classifiable as to human carcinogenicity), by the EPA/OPP Cancer Peer Review Committee in 1996.”).

<sup>164</sup> See IARC 2,4-D Monograph, *supra* note 14, at 108-26.

<sup>165</sup> EPA, Conducting a Human Health Risk Assessment - Dose Response, <https://www.epa.gov/risk/conducting-human-health-risk-assessment> (last visited Nov. 30, 2016).

people (intraspecies), and 10X juveniles being more sensitive than adults (FQPA)] is recommended.

**a. EPA incorrectly evaluated and dismissed evidence of thyroid toxicity and other endocrine effects**

NRDC is gravely concerned that EPA uses NOAELs that inadequately account for thyroid toxicity. In the assessment, EPA uses a developmental NOAEL of 25 mg/kg/day for acute dietary exposures in women of reproductive age and a NOAEL of 21 mg/kg/day for chronic dietary exposures for all populations. These levels do not adequately take into account thyroid toxicity made clear in the studies within the Agency's own record of adverse effects to the thyroid in adult and young animals. In particular, ample evidence for thyroid effects are clear from the Extended One Generation Reproductive Toxicity rat study,<sup>166</sup> the 90-day oral toxicity study in dogs,<sup>167</sup> a combined chronic toxicity/carcinogenicity study in rats,<sup>168</sup> and the chronic toxicity/carcinogenicity study with a chronic neurotoxicity screening battery substudy.<sup>169</sup> The thyroid is particularly important for the proper neurodevelopment of developing fetuses, infants, and children, therefore we recommend a significantly lower RfD, aPAD, and cPAD for all sensitive populations.

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<sup>166</sup> U.S. EPA. 2,4-D: Review of Extended 1-Generation Reproduction Study and Dose-Range Finding and Pharmacokinetic Titration Studies. 2010. EPA data evaluation record MRID:47972101

<sup>167</sup> U.S. EPA. Data Evaluation Report on Subchronic Toxicity in Dogs with 2,4-Dichlorophenoxyacetic acid. 1990. Data evaluation record MRID: 41737301

<sup>168</sup> Jeffries, TK, Yano, BL, Ormand, JR and Battjes, JE. "2,4-Dichlorophenoxyacetic Acid: Chronic Toxicity/Oncogenicity Study In Fischer 344 Rats-Final" The Toxicology Research Laboratory, Dow Chemical Co., Midland, Michigan. Study ID: K-002372-064. 3/28/95. MRID No. 43612001

<sup>169</sup> Mattsson J, Jeffries T, Yano B (1994b). 2,4-Dichlorophenoxyacetic acid: Chronic neurotoxicity study in Fischer 344 rats. Lab Project Number K-002372-064N, K-002372-064. Unpublished study prepared by The Dow Chemical Co. 1091 p. MRID: 43293901

Thyroid hormones are important for a myriad of bodily functions, including metabolism, proper maintenance of body temperature, differentiation of cell types within the body, and fetal and postnatal brain development. Chemicals that alter the tightly controlled thyroid system can cause devastating and irreversible changes during sensitive life stages, as EPA's own Science Advisory Board has noted.<sup>170</sup> The SAB's finding that "hypothyroxinemia (i.e., low levels of thyroid hormone) is a more appropriate indicator of the potential adverse health effects than the more pronounced decreases in thyroid hormone associated with hypothyroidism,"<sup>171</sup> suggests that even small changes in thyroid hormone levels (e.g., those resulting in sub-clinical thyroid disease) in the mother should be considered adverse for the developing brain of her fetus. In its evaluation of the Extended One Generation Reproductive Toxicity (EOGRT) study, EPA notes in its data evaluation record<sup>172</sup> that both offspring and dams exhibit low levels of the thyroid hormones T4 and T3 after exposure to 2,4-D, and increased levels of thyroid stimulating hormone (TSH) – all signs of thyroid toxicity.

There is ample evidence that 2,4-D is an endocrine disruptor. The adverse thyroid effects seen in the *in vivo* studies EPA cites, epidemiology studies showing increased hypothyroidism in farm workers exposed to 2,4-D,<sup>173</sup> decreased thyroid hormone (T4) production in zebrafish larva

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<sup>170</sup> EPA Science Advisory Board, SAB Advice on Approaches to Derive a Maximum Contaminant Level Goal for Perchlorate, 2013. See page 2 [http://yosemite.epa.gov/sab/sabproduct.nsf/0/86E44EE7F27EEC1A85257B7B0060F364/\\$File/EPA-SAB-13-004-unsigned2.pdf](http://yosemite.epa.gov/sab/sabproduct.nsf/0/86E44EE7F27EEC1A85257B7B0060F364/$File/EPA-SAB-13-004-unsigned2.pdf).

<sup>171</sup> *Id.*

<sup>172</sup> U.S. EPA. 2,4-D: Review of Extended 1-Generation Reproduction Study and Dose-Range Finding and Pharmacokinetic Titration Studies. 2010. EPA data evaluation record MRID: 47972191.

<sup>173</sup> Goldner WS, Sandler DP, Yu F, et al. Hypothyroidism and Pesticide Use Among Male Private Pesticide Applicators in the Agricultural Health Study. *Journal of Occupational and Environmental Medicine*. 2013;55(10):1171-1178. doi:10.1097/JOM.0b013e31829b290b.



exposed to 2,4-D,<sup>174</sup> along with the demonstrated ability of 2,4-D to bind to the thyroid receptor in *in vitro* experiments, indicate these effects. Tox21<sup>175</sup> data add to the weight of evidence, and add uncertainty to studies suggesting a lack of endocrine activity,<sup>176</sup> by revealing that 2,4-D is active in the thyroid receptor antagonist assay (as well as in the estrogen, androgen, and arylhydrocarbon receptor assays).<sup>177</sup> EPA is thus not justified in dismissing evidence of thyroid toxicity in its hazard evaluation.

Furthermore, EPA has improperly considered the levels at which these thyroid effects can occur. Specifically, the EOGRT study shows thyroid effects at multiple doses:

<b>Table 1. Thyroid Hormone- GD 17 Satellite Females</b>				
Parameter	0 ppm	100 ppm	300 ppm	600 ppm
Females				
N=	11	10	10	12
T3 (ng/dL)	73.12±14.17	71.69±11.42	69.64±10.67 ↓5%	68.12±19.04 ↓7%
Range	52.83-95.78	53.99-93.33	47.94-89.35	35.95-104.12
T4 (µg/dL)	1.26±0.28	1.22±0.43	1.16±0.64 ↓8%	1.15±0.42 ↓9%
Range	0.82-1.66	0.77-1.42	0.46-2.17	0.61-1.87
TSH(ng/mL)	2.92±1.56	2.76±0.84	2.60±1.37	3.65±1.59 ↑25%
range	1.03-5.62	1.47-4.02	1.17-3.45 (6.01) <sup>A</sup>	1.25-6.23 <sup>B</sup>
#>4	2	1	1 <sup>A</sup>	5
Data from Table 59, page 305 of study report; <sup>A</sup> identified as outlier in report; <sup>B</sup> not identified as outlier in report. (Data Evaluation Record MRID: 43293901; Table 21, page 42-3)				
<b>Table 2. Thyroid Hormone – F1 PND 4 (culled) pups</b>				

<sup>174</sup> Raldua D, Babin PJ. Simple, Rapid Zebrafish Larva Bioassay for Assessing the Potential of Chemical Pollutants and Drugs to Disrupt Thyroid Gland Function. *Environmental Science & Technology*. 2009;43(17):6844-6850. doi:10.1021/es9012454.

<sup>175</sup> A multi-agency collaborative (including EPA) aimed at investigating the use of cost effective, high-throughput, *in vitro* technologies to screen and predict the toxicity of large numbers of untested chemicals (<http://www.epa.gov/ncct/Tox21/>; last visited June 15, 2014)

<sup>176</sup> Coady KK, Lynn Kan H, Schisler MR, et al. Evaluation of potential endocrine activity of 2,4-dichlorophenoxyacetic acid using *in vitro* assays. *Toxicology in vitro : an international journal published in association with BIBRA*. 2014;28(5):1018-25. doi:10.1016/j.tiv.2014.04.006.

<sup>177</sup> Data accessed via <http://actor.epa.gov/dashboard/>

Parameter	0 ppm	100 ppm	300 ppm	600/800 ppm
Males				
T3 (ng/dL)	34.51±5.83 (9)	35.90±5.99 (8)	35.46±7.07 (8)	32.19±8.17 ↓7%
T4 (µg/dL)	0.64±0.25	0.62±0.32 (8)	0.55±0.21 ↓14%	0.56±0.19 ↓12%
TSH(ng/mL)	1.12±0.31	0.98±0.26 (7)	1.06±0.27 (9)	1.09±0.20
Females				
T3 (ng/dL)	41.99±9.78	38.64±8.28 ↓8%	36.59±7.15 (6) ↓13%	40.29±8.06 (9)
T4 (µg/dL)	0.85±0.16	0.99±0.16	0.72±0.32 (9) ↓15%	0.73±0.23 ↓14%
TSH(ng/mL)	0.97±0.24	1.06±0.35 ↑9%	0.90±0.18 (9)	1.15±0.34 ↑19%
Data from Tables 73 and 74, pages 324 and 325 of report; n=10, unless ( ) (Data reproduced from Data Evaluation Record MRID: 43293901 ; Table 31, page 48)				

Table 3. Thyroid Hormone – F1 PND 22 Weanlings				
Parameter	0 ppm	100 ppm	300 ppm	600/800 ppm
Males				
T3 (ng/dL)	107.22±16.59	100.82±12.88	86.56±9.16* ↓19%	93.46±15.06 ↓13%
T4 (µg/dL)	3.62±0.84	4.40±0.54	2.98±0.75	2.59±1.04* ↓28%
TSH(ng/mL)	1.32±0.24	1.25±0.59	1.48±0.73 ↑12%	1.27±0.37
Females				
T3 (ng/dL)	99.14±14.68	110.43±20.01	99.42±15.02	107.42±11.68
T4 (µg/dL)	3.57±0.75	3.99±1.19	3.55±0.77	2.85±0.66 ↓20%
TSH(ng/mL)	0.99±0.26	1.13±0.33	0.94±0.22	1.02±0.16
Data from Tables 75 and 76. Pages 326 and 327 of report; n=10; * α=0.05 Males not given adult dietary concentration until PND 35. (Data reproduced from Data Evaluation Record MRID: 43293901 ; Table 32, page 49)				

Table 4. Thyroid Hormone – F1 Set 1a Males (PND 62-64)				
parameter	0 ppm	100 ppm	300 ppm	600/800 ppm
Males				
T3 (ng/dL)	78.69±12.07	69.78±7.91	66.77±9.69	72.03±17.40
range	63.03-102.07	58.34-80.05	46.97-84.54	43.51-94.29
T4 (µg/dL)	4.75±0.92	4.46±1.23	5.31±1.09 ↑12%	4.11±0.85 ↓13%
Range	3.50-6.44	2.93-6.56	2.79-6.35	2.63-5.45
TSH(ng/mL)	2.95±0.74	3.21±1.29	3.72±0.97 ↑26%	3.62±1.20 ↑23%
Range	2.12-3.42 (4.73) <sup>A</sup>	1.91-5.66	2.77-5.26	2.11-5.77
Females				
T3 (ng/dL)	67.08±17.71	66.89±10.71	70.45±12.99	74.28±14.67
Range	51.49-81.31 (109.79) <sup>A</sup>	46.65-79.02	57.25-96.84	↑11% 58.13-100.28
T4 (µg/dL)	2.35±±1.05	2.27±0.85	2.80±1.43 ↑19%	2.79±1.08 ↑19%
Range	1.00-4.72	0.99-3.36	1.54-5.65	1.29-5.03

TSH(ng/mL) Range	1.89±0.53 0.93-2.60	2.05±0.61 1.24-3.00	2.10±0.42 ↑11% 1.84-2.46 (1.03) <sup>A</sup>	2.34±0.67 ↑24% 1.66-2.41 (4.15) <sup>A</sup>
Data from Tables 77 and 78, pages 328 and 329 of report; n=10; <sup>A</sup> outlier (Data reproduced from Data Evaluation Record MRID: 43293901 ; Table 33, page 50)				

Despite ample evidence showing significant, and in some cases sustained, changes in thyroid hormone production at 2,4-D dose levels as low as 100 ppm, EPA incorrectly selected 600/800 ppm as a NOAEL and inappropriately ignored thyroid effects.

NRDC has two concerns about this determination. First, a NOAEL of 600/800 ppm cannot be justified because thyroid effects occurred at lower levels. Second, thyroid effects are reported at the lowest dose tested, 100 ppm (5 mg/kg/day). Thus, the 100 ppm dose can be used appropriately only to develop a LOAEL.

EPA selected the highest dose, 600/800 ppm, as its point of departure by dismissing observed thyroid effects at lower levels as adaptive rather than adverse. Such a determination is flawed because it relies too heavily on a traditional monotonic dose-response relationship paradigm (e.g., the dose makes the poison and dose-response curves must be monotonic) to define the association between 2,4-D exposure and thyroid outcomes.<sup>178</sup> Current scientific understanding of hormone disrupting chemicals do not support this rationale and suggest, instead, that EPA should consider the possibility that 2,4-D dose-response curves are nonmonotonic. The National Academies of Sciences in its *Review of the Environmental Protection Agency's State-of-the-Science Evaluation of Nonmonotonic Dose-Response Relationships as they Apply to Endocrine Disruptors*<sup>179</sup> wrote that EPA should use caution with

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<sup>178</sup> U.S. EPA. 2,4-D: Review of Extended 1-Generation Reproduction Study and Dose-Range Finding and Pharmacokinetic Titration Studies. 2010. EPA data evaluation record MRID: 47972191 at 6.

<sup>179</sup> *Review of the Environmental Protection Agency's State-of-the-Science Evaluation of Nonmonotonic Dose-Response Relationships as They Apply to Endocrine Disruptors*. The

concepts like adaptation because “effects that are adaptive in some people are adverse in others.”<sup>180</sup> The NAS additionally notes that, “consideration should be given to potential windows of susceptibility (for example, during fetal development), sensitive populations (for example, those with pre-existing health conditions), and other factors (such as multiple chemical exposures) in making these [adaptive versus non-adaptive] distinctions.”<sup>181</sup> EPA’s blatant disregard for the current scientific understanding of dose-response in endocrine disruption leads it to a wholly insufficient point of departure for 2,4-D.

On the basis of this evidence, EPA must use, at a minimum, the more protective 100 ppm (5 mg/kg/day) and 10X uncertainty factor for LOAEL to NOAEL extrapolation as the Point of Departure. To calculate the Population Adjusted Dose (PAD), the PoD should be adjusted further with the standard 10X for animal to human extrapolation (interspecies), 10X for differences between people (intraspecies), and 10X for juveniles being more sensitive than adults (FQPA).<sup>182</sup>

### **iii. EPA’s exposure estimates are insufficient to protect sensitive populations**

In addition to underestimating the toxicity of 2,4-D, EPA has underestimated the higher frequencies of exposure that will occur with expanded use in this new formulation, as well as important potential exposure pathways. These flawed exposure estimates, combined with RfDs and PADs that are not sufficiently protective, leave pregnant women, fetuses, infants, and children particularly vulnerable to 2,4-D’s toxic effects.

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National Academies Press; 2014. Available at:  
[http://www.nap.edu/openbook.php?record\\_id=18608](http://www.nap.edu/openbook.php?record_id=18608).

<sup>180</sup> *Id.* at 7.

<sup>181</sup> *Id.*

<sup>182</sup> U.S. EPA. 2,4-D. Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean. 2013. See Table 4.5.4.1, p. 18.

**a. EPA's dietary risk assessments do not adequately capture all reasonable exposure risks**

In its calculation of acute and chronic dietary risk estimates, EPA did not clearly articulate the assumptions made in calculating exposures for various sub-populations. We are especially concerned that EPA labels both the acute and chronic dietary assessments “unrefined,”<sup>183</sup> which appear to be rough estimates that may be inaccurate given a lack of data on key variables such as anticipated residues. EPA's proposal is insufficiently transparent to determine the nature and extent of data gaps leading to its “unrefined” estimate, and it is thus impossible for NRDC, or other members of the public, to determine whether the resulting proposal adequately protects human health.

**b. EPA does not properly consider drinking water exposure**

EPA states in the *Environmental Fate and Effects Division's Risk Assessment for the Reregistration Eligibility Document for 2,4-Dichlorophenoxyacetic Acid*,<sup>184</sup> that EPA SCIGROW groundwater models do not accurately predict maximum concentrations of 2,4-D in groundwater. Also, by its own admission, EPA's use of the maximum monitored National Water Quality Assessment (NAWQA) concentration in the proposal is also likely to be an underestimate because the USGS and EPA monitoring data come from “non-targeted” sampling areas. EPA specifically states:

EFED has determined that the available monitoring data is non-targeted to 2,4-D use because it was not collected with the intention of capturing maximum acute and chronic 2,4-D concentrations. Targeted monitoring data should be collected with a sampling

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<sup>183</sup> *Id.* at 25 (acute) and 26 (chronic)

<sup>184</sup> U.S. EPA. Environmental Fate and Effects Division's Risk Assessment for the Reregistration Eligibility Document for 2,4-Dichlorophenoxyacetic Acid. See page 52 (<http://www.epa.gov/espp/litstatus/effects/24d/attachment-b.pdf>; last visited June 25, 2014)

frequency designed to capture peak runoff events coinciding with a specific pesticide use, with a duration designed to provide sufficient data to estimate long term exposures, and be specifically tailored to the individual geography and crop uses of the target pesticide. The monitoring data used in this assessment, while plentiful and of high quality, was not collected specifically with 2,4-D use in mind and is therefore considered to be non-targeted to 2,4-D use but was used in this assessment for comparison against model predictions.<sup>185</sup>

In fact, there is reason to believe that EPA's selection of 15 ppb as a maximum value is flawed. Though the Agency dismissed its STORET monitoring data due to concerns about QA/QC that it did not describe, STORET monitoring has detected a peak groundwater concentration of 7500 µg ae/L,<sup>186</sup> giving rise to additional uncertainty in the adequacy of EPA's presumed groundwater concentrations. Moreover, as the EFED summary above indicates, water concentrations that would occur during peak runoff are likely to be far higher than detected using standard non-targeted monitoring, adding to the likely underestimate and additional uncertainty. The groundwater concentration of 15 ppb used by EPA for its dietary risk assessment is, therefore, likely to have missed higher concentrations that could be found in areas with high 2,4-D use.

These underestimates pose particular risk in the ten additional states where EPA proposes to allow the use of this new herbicide given the heightened reliance on groundwater as a drinking water source in this region. In the proposed use states, 86% of all the public water systems in these ten states (60% in Arkansas, 64% in Kansas, 93% in Louisiana, 98% in Minnesota, 92% in Missouri, 99% in Mississippi, 94% in Nebraska, 55% in Oklahoma, 60% in Tennessee, and 72%

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<sup>185</sup> *Id.* at 42

<sup>186</sup> *Id.*

in North Dakota) rely on ground water as their primary source of water,<sup>187</sup> and 10% of the groundwater withdrawals in the proposed states (2% in Arkansas, 6% in Kansas, 25% in Louisiana, 52% in Minnesota, 17% in Missouri, 18% in Mississippi, 4% in Nebraska, 25% in Oklahoma, 75% in Tennessee, and 29% in North Dakota) being made by public and domestic wells in 2005.<sup>188</sup> Approximately 43 million people in the United States rely on domestic wells for their drinking water supply, making them an important vehicle for groundwater exposure.<sup>189</sup>

EPA's surface water estimates also seem insufficiently protective, in that they are also based on models trained with non-targeted 2,4-D monitoring data. EFED surface water modeling revealed peak acute exposures of up to 4000 µg ae/L for some use scenarios and indicated that setback distances of up to 1500 feet were used in some calculations.<sup>190</sup> Setback distances, or the distance a pesticide can be applied near a drinking water source, are specified on the pesticide label. EPA states that a 2,4-D Master Label was the source of its application assumptions,<sup>191</sup> but publically available copies of the Master Label do not include 2,4-D choline salt and the expanded.<sup>192</sup>

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<sup>187</sup> SDWISFED GPRA data (7/1/2012 to 6/30/2013); <http://water.epa.gov/scitech/datait/databases/drink/sdwisfed/pivottables.cfm>

<sup>188</sup> Kenny, J.F., Barber, N.L., Hutson, S.S., Linsey, K.S., Lovelace, J.K., and Maupin, M.A., 2009, Estimated use of water in the United States in 2005: U.S. Geological Survey Circular 1344, Table 4A, p. 11; <http://pubs.usgs.gov/circ/1344/pdf/c1344.pdf>

<sup>189</sup> <http://www.usgs.gov/newsroom/article.asp?ID=2173#.U6OYTPlDWSO>

<sup>190</sup> U.S. EPA. Environmental Fate and Effects Division's Risk Assessment for the Reregistration Eligibility Document for 2,4-Dichlorophenoxyacetic Acid. See page 42 (<http://www.epa.gov/espp/litstatus/effects/24d/attachment-b.pdf>; accessed 11/08/2014)

<sup>191</sup> *Id.* at 41.

<sup>192</sup> <http://www.epa.gov/oppfead1/endanger/litstatus/effects/redleg-frog/2-4-d/appendix-n.pdf>.



**c. EPA does not clearly demonstrate that breast milk is adequately considered in dietary exposure for infants**

In its human health risk assessment, EPA states that “toxicokinetic studies conducted in pregnant rats show that 2,4-D is transferred through maternal milk to the pups”<sup>193</sup>, and has been shown to impact the nutritional content of the milk.<sup>194</sup> Thus, maternal exposure to 2,4-D and subsequent transfer through breast milk may be a significant contributor to dietary 2,4-D exposure in infants. It is absolutely critical that EPA properly and adequately assess the levels of 2,4-D that could be passed to infants via both breast milk and formula (via water used to reconstitute formula and via the formula itself), and without transparent information to evaluate EPA assumptions about infant consumption amounts of 2,4-D via breast milk, it is unclear that developing brains are sufficiently protected.

**d. EPA has not appropriately accounted for the volatility of 2,4-D in its exposure risk calculations**

In its volatilization modeling and risk assessment evaluation of 2,4-D, EPA states that its field volatility study (i.e. its monitoring data) indicated that volatilization of 2,4-D from treated crops does occur and could result in bystander exposure to vapor phase 2,4-D.<sup>195</sup> However, the Agency dismisses this data and relies upon modeling estimates derived from its Probabilistic

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<sup>193</sup> U.S. EPA. 2,4-D. Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean. 2013. p.10.

<sup>194</sup> Sturtz N, Bongiovanni B, Rassetto M, Ferri A, de Duffard AME, Duffard R. Detection of 2,4-dichlorophenoxyacetic acid in rat milk of dams exposed during lactation and milk analysis of their major components. *Food Chem Toxicol.* 2006;44(1):8-16. doi:10.1016/j.fct.2005.03.012.

<sup>195</sup> U.S. EPA. 2,4-D. Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean. 2013. p. 34.

Exposure and Risk model for FUMigants (PERFUM) instead. EPA's modeling exercise indicates that airborne concentrations are "not of concern."<sup>196</sup>

It is not clear why EPA decided to reject monitoring data and use modeling estimates in its place. Again, the public is not provided adequate information to evaluate the Agency's determination on this potentially critical exposure pathway. In fact, EPA sheds doubt on the modeling results, stating that some information "could be excluded" in its volatilization models given "limited information available and a lack of intentional statistical design to quantitatively evaluate [factors that could potentially affect the emission rates and off-site transport of 2,4-D]."<sup>197</sup> However, EPA does not make clear in its proposal which information it is excluding. As a consequence, it is impossible to determine whether the Agency used reasonable worst-case assumptions and/or adequately protective cut off figures in its probabilistic estimation. In addition, volatilization models should include estimates or uncertainty factors for additional off-gassing emissions, with particular concern for additional flux associated with use of 2,4-D later in the growing season (potential for mid-to-late July<sup>198</sup> for corn and mid-to-late June for soy<sup>199</sup>), increased use and temperature factors associated with climate change,<sup>200</sup> and should include location-based, worst-case scenario weather information for the proposed states in the PERFUM

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<sup>196</sup> *Id.*

<sup>197</sup> *Id.*

<sup>198</sup> <http://www.sdstate.edu/ps/extension/crop-mgmt/corn/upload/Corn-growth-stage-day-and-GDU-calendar10.pdf> and <http://www.sdstate.edu/ps/extension/crop-mgmt/corn/upload/Corn-growth-stage-day-and-GDU-calendar10.pdf>; last visited June 15, 2014.

<sup>199</sup> <http://www.extension.umn.edu/agriculture/soybean/soybean-growth-and-development-information-for-replant-decisions/>; last visited June 17, 2014.

<sup>200</sup> Ziska LH. Increasing Minimum Daily Temperatures Are Associated with Enhanced Pesticide Use in Cultivated Soybean along a Latitudinal Gradient in the Mid-Western United States. *PloS one*. 2014;9(2):e98516. doi:10.1371/journal.pone.0098516.

model to ensure that the highest risk estimates are used. From the public information available, it does not appear that EPA has taken the higher frequencies of application later in the season (at warmer temperatures) into account with its modeling.

**e. EPA has used invalid assumptions in determining the exposure risk estimates for 2,4-D spray drift**

In its assessment of the potential for population exposure from spray drift, EPA inappropriately bases its analysis on the “premise of compliant applications” in accordance with label restrictions.<sup>201</sup> As we have raised in prior NRDC comments (APPENDIX B),<sup>202</sup> EPA does not collect user testing data to evaluate whether applicators understand and routinely implement the control measures that would limit spray drift. The presence of 2,4-D in homes<sup>203</sup> and the environment suggest either that non-compliant applications are occurring or that the labels are insufficiently protective. EPA’s assumption of compliant application falls far short of a reasonable worst-case assessment of harm that would be caused by a variety of very common problems such as poor user understanding of the local factors that influence spray drift (e.g., wind speed and meteorological conditions), poor worker training, and a host of additional real-world problems that lead to spray drift exposures. In the absence of data to support its compliant application assumption, EPA should assume a reasonable worst-case scenario of non-compliance in its calculations of drift and volatilization.

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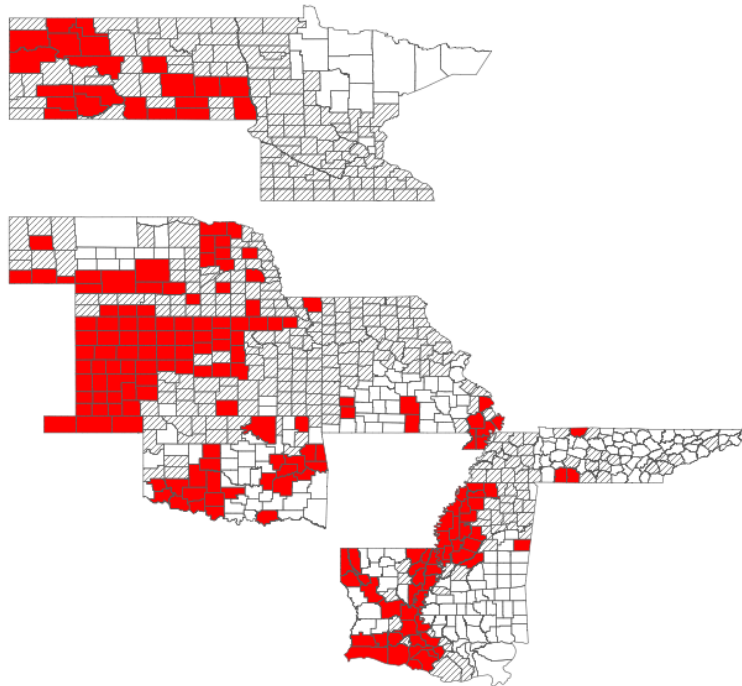
<sup>201</sup> U.S. EPA. 2,4-D. Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean. 2013. See Table 4.5.4.1, p. 35.

<sup>202</sup> Sass J, Comments on two draft guidance documents describing how off-site spray drift will be evaluated for ecological and human health risk assessments for pesticides. April 30, 2014. EPA-HQ-OPP-2013-0676

<sup>203</sup> Morgan MK, Sheldon LS, Thomas KW, et al. Adult and children’s exposure to 2,4-D from multiple sources and pathways. *J Expo Sci Environ Epidemiol*. 2008;18(5):486-494. doi:10.1038/sj.jes.7500641.

Additionally, EPA estimates drift based on an existing *turf* drift assessment, incorrectly concluding that “[i]f the maximum application rate on crops adjusted by the amount of drift expected is less than or equal to existing turf application rates, the existing turf assessment is considered protective of spray drift exposure.”<sup>204</sup>

The increased exposure to Enlist Duo will occur through an increased *frequency* of application, not necessarily an increased application rate. By again neglecting to account for increased frequency of application inherent in the proposed uses of Enlist Duo, EPA misses additional windows of exposure that are the heart of the matter. While 2,4-D application is limited to 1.5 lb ae/acre/year on turf, Enlist Duo seeks approval for an application rate of 3 lb ae/acre/season. Increased frequency of exposure, particularly over large geographic areas (Figure 1), could be detrimental to the health and well-being of pregnant women and children.



**Figure 1.** Potential areas of geographic expansion of 2,4-D with Enlist Duo registration. Counties mapped in red represent areas with more than 10,000 lbs of 2,4-D usage in 2011 (data from USGS 2011 ; ref 1). Counties mapped in hatched lines represent counties with more than 10,000 pounds of glyphosate usage in 2011 (data from USGS 2011; ref 1). Enlist Duo™ (a combination of 2,4-D and glyphosate) could significantly expand the use of 2,4-D in the cross-hatched areas.

on Herbicide Tolerant Corn and Soybean. 2013. See Table 4.3.4.1, p. 33.

**iv. EPA must use a minimum of the statutorily required FQPA tenfold safety factor to protect infants and children from the toxic effects of 2,4-D**

EPA is required to use a 10X safety factor “for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children.”<sup>205</sup> EPA may use a different safety factor “*only if*, on the basis of reliable data, such margin will be safe for infants and children.”<sup>206</sup>

In the proposed registration of 2,4-D, EPA contends that the FQPA safety factor can be reduced from 10X to 1X because the “[t]he toxicity database is complete and adequate to assess safety for infants and children.”<sup>207</sup> As we illustrated in our discussion of thyroid toxicity, the 1-generation study EPA relied on to determine thyroid effects did not determine a NOAEL for 2,4-D. The increased susceptibility of offspring to 2,4-D toxicity, the ability of 2,4-D to bind the thyroid, androgen, estrogen, and arylhydrocarbon receptors in *in vitro* Tox21 assays, human epidemiology demonstrating thyroid effects, and the uncertainty of the shape of the dose curve alone, justify the retention of, at a minimum, the statutorily-presumed children’s 10X uncertainty factor to be protective of pregnant women, infants, and children.

In addition to specific thyroid-related effects, the peer-reviewed publication record contains several additional studies that are relevant to adverse effects on fetal development and hormone regulation, including (but not limited to):

- Kooijman R, Devos S, Hooghe-Peters E. Inhibition of in vitro cytokine production by human peripheral blood mononuclear cells treated with xenobiotics: Implications for the

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<sup>205</sup> 21 U.S.C. § 346a(b)(2)(C)

<sup>206</sup> *Id.*

<sup>207</sup> U.S. EPA. 2,4-D. Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean. 2013. See page 14

prediction of general toxicity and immunotoxicity. *Toxicology in Vitro*. 2010;24(6):1782-1789. doi:10.1016/j.tiv.2010.07.007.

- Stebbins-Boaz B, Fortner K, Frazier J, et al. Oocyte maturation in *Xenopus laevis* is blocked by the hormonal herbicide, 2,4-dichlorophenoxy acetic acid. *Molecular Reproduction and Development*. 2004;67(2):233-242. doi:10.1002/mrd.10396.
- Cavieres MF, Jaeger J, Porter W. Developmental toxicity of a commercial herbicide mixture in mice: I. Effects on embryo implantation and litter size. *Environmental Health Perspectives*. 2002;110(11):1081-1085.
- Pochettino AA, Bongiovanni B, Duffard RO, Evangelista de Duffard AM. Oxidative stress in ventral prostate, ovary, and breast by 2,4-dichlorophenoxyacetic acid in pre- and postnatal exposed rats. *Environmental Toxicology*. 2013;28(1):1-10. doi:10.1002/tox.20690.
- Ferri A, Duffard R, Sturtz N, de Duffard AME. Iron, zinc and copper levels in brain, serum and liver of neonates exposed to 2,4-dichlorophenoxyacetic acid. *Neurotoxicology and Teratology*. 2003;25(5):607-613. doi:10.1016/S0892-0362(03)00075-8.
- Greenlee AR, Ellis TM, Berg RL. Low-dose agrochemicals and lawn-care pesticides induce developmental toxicity in murine preimplantation embryos. *Environmental Health Perspectives*. 2004;112(6):703-709. doi:10.1289/ehp.6774.

The published literature, as well as the thyroid effects mentioned above, provide multiple streams of evidence that warrant a 10X FQPA uncertainty factor necessary to protect the health of pregnant mothers, infants, and children.

Additionally, immense uncertainty remains in the exposure risk estimates calculated by EPA. As stated in our exposure estimate sections above, EPA lacks considerable data to accurately estimate exposure to 2,4-D from the diet (including food and water contributions), breast milk, volatilization, and spray drift sources for pregnant women, infants, and children, and thus must rely on reasonable worst case estimations. These additional uncertainties provide an additional clear rationale for retaining, at a minimum, the 10X FQPA children's protection uncertainty factor.

Thus, EPA must retain, at a minimum, the statutorily-presumed FQPA 10x uncertainty factor to take into account potential pre-and post-natal toxicity and completeness of the data with respect to infants and children.

**D. FIFRA requires EPA to assess the serious risks that Enlist Duo poses to monarchs, and to adequately assess Enlist Duo’s human health risks, relying on current science and data**

**1. EPA must consider harm to monarch butterflies**

Before registering Enlist Duo as a new pesticide, EPA is required to review “all relevant data in the possession of the Agency,” 40 C.F.R. § 152.112(b), determine “that no additional data [were] necessary,” *id.* § 152.112(c), and ultimately determine that Enlist Duo will not cause “unreasonable adverse effects on the environment,” 7 U.S.C. § 136a(c)(5)(C), (D); *accord* 40 C.F.R. § 152.112(e). EPA’s evaluation of Enlist Duo—and its component ingredients, glyphosate and 2,4-D—ignore all evidence of harm to monarch butterflies arising from the herbicide’s destruction of milkweed.

**i. EPA has refused to consider an entire body of scientific literature demonstrating that Enlist Duo poses a serious risk to monarch butterflies**

As discussed, a significant body of scientific studies concludes that use of herbicides, particularly those containing glyphosate, on herbicide-resistant crops has been a driving force behind monarch population decline. Enlist Duo is specifically intended to suppress milkweed,<sup>208</sup> which the monarch population needs to survive. In addition, Enlist Duo’s intended pairing with Enlist corn, soybeans, and cotton that are resistant to Enlist Duo, enables use of the herbicide frequently, at high volumes, and during the milkweed’s most vulnerable flowering stage.<sup>209</sup> EPA predicts that Enlist Duo will extend the viability of glyphosate for herbicidal use, and recognizes

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<sup>208</sup> EPA, *supra* note 8 (stating that Enlist Duo can be used “[f]or suppression” of common milkweed).

<sup>209</sup> CFS 2014 Comments, *supra* note 19, at 13.



that Enlist Duo will likely cause 2,4-D use to increase.<sup>210</sup> According to the Department of Agriculture, EPA's registration of Enlist Duo will increase the use of 2,4-D up to six-fold.<sup>211</sup> Monarch experts agree that it is precisely the type of activity proposed here—the application of herbicides to herbicide-resistant crops—that has been a leading cause of the monarch's stark decline over the past two decades.<sup>212</sup>

Information on Enlist Duo's potential to harm monarch butterflies is thus both “relevant,” 40 C.F.R. § 152.112(b), and “necessary,” *id.* § 152.112(c), to determining whether registration of Enlist Duo would cause “unreasonable adverse effects on the environment,” 7 U.S.C. § 136a(c)(5)(C), (D). *See* 40 C.F.R. § 158.75. Yet, EPA has thus far refused to consider that information.<sup>213</sup> Indeed, EPA has never considered, as part of *any* pesticide registration, the impacts that either of Enlist Duo's active ingredients has on monarchs. Without analyzing how Enlist Duo would impact milkweed and monarchs, EPA lacks an adequate basis to conclude that registration of Enlist Duo will not cause unreasonable adverse effects on the environment, as required by FIFRA.

**ii. EPA has never previously considered impacts to monarchs and thus cannot rely exclusively on its previous risk assessments for glyphosate**

In its initial registration decision for Enlist Duo, EPA reasoned that it did not need to conduct any new risk assessments for glyphosate, because registration of Enlist Duo ostensibly

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<sup>210</sup> Phillips, *supra* note 19, at 8-9.

<sup>211</sup> APHIS, *supra* note 21, at x.

<sup>212</sup> *See, e.g.,* Flockhart, *supra* note 98, at 1, 7-8; CFS 2014 Comments, *supra* note 19, at 4; Pleasants & Oberhauser, *supra* note 48, at 1.

<sup>213</sup> *See* EPA, 2014 Response to Public Comments, at 23.

would not result in any new use of glyphosate.<sup>214</sup> EPA has reiterated that reasoning in its new Proposed Registration Decision.<sup>215</sup>

This approach violates FIFRA's registration requirement, because it disregards the fact that EPA has *never* considered impacts to monarch butterflies when previously registering any glyphosate-containing pesticide. The strong body of science demonstrating the link between monarch decline and herbicide use on herbicide-resistant crops emerged after 1993, the last time EPA re-registered glyphosate-based pesticides.

Moreover, the agency cannot rely on an unsupported assumption that the total amount of glyphosate used will remain constant to conclude that the glyphosate in Enlist Duo will not harm monarchs.<sup>216</sup> EPA explicitly did not evaluate how Enlist Duo's registration would affect "total loading of herbicides."<sup>217</sup> Contrary to the agency's assumption, glyphosate's decreasing efficacy strongly suggests that reliance on glyphosate-containing pesticides will decrease but for EPA's registration of Enlist Duo.<sup>218</sup> And the information available indicates that Enlist Duo may even prompt growers to expand their reliance on glyphosate-containing pesticides.<sup>219</sup> Either way,

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<sup>214</sup> See EPA, Final Registration Decision for Enlist Duo Herbicide, at 1 (Oct. 14, 2014); see also EPA Opp. to Mot. to Stay, *NRDC v. EPA*, Case No. 14-73353, ECF No. 24, at 14 (9th Cir. Jan. 23, 2015) ("[T]he registration does not change the lawful scope of glyphosate use, and EPA properly relied on its prior assessments and existing glyphosate registrations in finding that the glyphosate portion of Enlist Duo will not cause 'unreasonable adverse effects on the environment.'" (quoting 7 U.S.C. § 136a(c)(5))).

<sup>215</sup> EPA, Proposed Registration Decision, at 2.

<sup>216</sup> Compare Phillips, *supra*, note 19, at 9, with EPA, 2014 Response to Public Comments, at 32.

<sup>217</sup> Phillips, *supra*, note 19, at 9.

<sup>218</sup> See *id.* at 8; *supra* Section III.A.

<sup>219</sup> See *supra* Section III.A.

Enlist Duo's registration would harm milkweed and monarchs by perpetuating or expanding heavy glyphosate use.

EPA may certainly consider data submitted to support older pesticide applications when registering a new pesticide (provided certain conditions in the statute are met). *See* 7 U.S.C. § 136a(c)(1)(F). But that does not mean that EPA may consider *only* the data submitted with older pesticide applications, ignoring all other relevant information properly before the agency. EPA itself, in its response to comments on Enlist Duo, acknowledged that “[p]roposed new registrations are held to the most current data requirements and up-to-date risk assessment practices.”<sup>220</sup> It would be unlawful for EPA to defy this principle and rely on an incomplete and outdated set of data, turning a blind eye to overwhelming new evidence that Enlist Duo will harm monarchs.

**iii. EPA's duty to ensure the safety of Enlist Duo at the time of registration is independent from, and additional to, its duty to ensure the continued safety of previously registered pesticides containing glyphosate**

In its response to public comments on its previous registration of Enlist Duo, EPA attempted to justify its failure to consider Enlist Duo's effects on monarch butterflies by explaining that the agency plans to evaluate glyphosate's effects on monarchs at some point in the future, as part of its registration review for glyphosate-containing pesticides.<sup>221</sup> This approach violates FIFRA. The statute provides multiple mechanisms for re-evaluating the safety of pesticides that have already been registered, *see, e.g.*, 7 U.S.C. § 136a(g) (registration review); *id.* § 136a(c)(8) (interim administrative review), but that does not relieve EPA of the

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<sup>220</sup> EPA, 2014 Response to Public Comments, at 25.

<sup>221</sup> *Id.* at 25, 27.

responsibility to make an initial safety determination for new pesticides based on all relevant information. Simply put, EPA may not register a new pesticide, acknowledge major unanswered questions about harm, and announce that it will consider those questions later, after the pesticide is already on the market. *See* 7 U.S.C. § 136a(c)(5)(C), (D).

Moreover, whether glyphosate alone harms monarchs (the question EPA promises to consider later) is not the same as whether Enlist Duo does. Enlist Duo contains two active ingredients, glyphosate and 2,4-D, and registration of Enlist Duo will significantly increase use of 2,4-D, which also kills milkweed.<sup>222</sup> EPA did not provide any explanation for its failure to consider 2,4-D's impacts, through its destruction of milkweed, on monarchs.<sup>223</sup> Under FIFRA, EPA is required to consider all relevant evidence of environmental harm, including harm to monarchs, before registering a pesticide.

Notably, FIFRA requires EPA to assess harm to monarchs, even if EPA disagrees with NRDC regarding the extent of harm that Enlist Duo poses to monarchs. The extent of harm is a question for EPA to assess, and the agency cannot simply disregard the issue before registering Enlist Duo.

**2. EPA must consider current science regarding Enlist Duo's health risks, and must correct the errors in its human health assessment for 2,4-D**

**i. EPA cannot ignore the current science relevant to human health risks posed by Enlist Duo**

In proposing to register Enlist Duo, EPA has entirely failed to consider current scientific findings about glyphosate's human health effects, including its cancer risks and dietary risks. EPA's previous health assessments for glyphosate are outdated; over 3000 relevant studies have

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<sup>222</sup> *See* APHIS, *supra* note 21 at x; CFS 2014 Comments, *supra* note 19, at ER 14-15.

<sup>223</sup> *See* EPA, 2014 Response to Public Comments, at 23.

been published since EPA re-registered glyphosate-containing pesticides in 1993, providing a basis for setting much more stringent exposure limits for glyphosate. New food residue data has also emerged since 1993, indicating that dietary exposures to glyphosate may be much higher than scientists previously believed. EPA must complete an updated human health risk assessment for glyphosate, without which the agency cannot properly find that Enlist Duo will not cause unreasonable adverse effects on human health or the environment. But in proposing to register Enlist Duo, EPA performed “no new assessment” for glyphosate, and the agency’s previous response to comments entirely ignored new evidence of the chemical’s human health risks.<sup>224</sup>

In March 2015, IARC determined that glyphosate is “probably carcinogenic to humans.”<sup>225</sup> Although NRDC and others notified EPA of this cancer finding before the agency issued its amended registration of Enlist Duo, the agency by its own admission “expressly declined to revisit any human health risk issues” in its expanded registration decision in 2015.<sup>226</sup> EPA’s conclusion that the glyphosate in Enlist Duo will not harm human health is thus based on outdated science and an agency review of that science conducted over twenty years ago.<sup>227</sup>

Similarly, with respect to 2,4-D, EPA disregarded IARC’s finding of possible carcinogenicity, simply stating that “2,4-D has been classified as a Category D chemical, i.e., not

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<sup>224</sup> EPA, Proposed Risk Assessment, at 2; *see* EPA, 2014 Response to Public Comments, at 23-25.

<sup>225</sup> Guyton, *supra* note 143, at 491.

<sup>226</sup> EPA’s Opp’n to Mot. to Stay, *NRDC v. EPA*, Case No. 15-71213, ECF No. 29-1, at 17 (9th Cir. June 22, 2015); *see* EPA, Decision to Amend Enlist Duo Herbicide Label to include additional states: Arkansas, Kansas, Louisiana, Minnesota, Missouri, Mississippi, Nebraska, Oklahoma, and North Dakota, at 5-6 (Mar. 31, 2015).

<sup>227</sup> *See* 1993 Glyphosate R.E.D. Facts, *supra* note 5, at 2-4; 1993 Glyphosate R.E.D., *supra* note 8, at 10-30; Jane Smith, EPA, Memorandum re The HED Chapter of the Reregistration Eligibility Document (RED) for Glyphosate, Case #0178, at 1-18 (Jan. 15, 1993).

classifiable as to human carcinogenicity. A quantitative cancer risk assessment is not required.”<sup>228</sup> But this classification was made two decades ago, back in 1996.<sup>229</sup> In light of IARC’s recent cancer finding for 2,4-D, and the post-1996 studies incorporated into IARC’s 2,4-D monograph, it would be inappropriate for EPA to rely on its outdated carcinogenicity classification for 2,4-D in registering Enlist Duo.

Such willful ignorance would violate FIFRA. EPA may not register a pesticide if it poses an unreasonable health risk to people. 7 U.S.C. §§ 136a(c)(5)(D), 136(bb). FIFRA regulations require EPA to consider data “sufficient to evaluate the potential of the product to cause unreasonable adverse effects on man,” 40 C.F.R. § 158.75, including “all relevant data” in the agency’s possession, *id.* § 152.112(b). Such data include evidence of cancer risk that has been published in the last twenty-five years.

In light of the current science, the studies on which EPA previously relied to determine that glyphosate was sufficiently safe for humans—primarily, the studies EPA relied on when it re-registered glyphosate-based pesticides in 1993—are no longer adequate, decades later, to assess Enlist Duo’s health risks. Those studies predated IARC’s new finding by over two decades and preceded the publication of myriad relevant new studies assessing the links between glyphosate and human health harms, including cancer. There’s no indication that EPA has considered, in connection with its proposed registration decision for Enlist Duo, the post-1993

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<sup>228</sup> EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide Tolerant Cotton, at 5, 35 (2016), *available at* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0594-0009>; *accord* EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean, at 4, 37 (2013).

<sup>229</sup> 2005 2,4-D R.E.D., *supra* note 16, at 19 (“2,4-D has been classified as a Category D chemical (i.e., not classifiable as to human carcinogenicity), by the EPA/OPP Cancer Peer Review Committee in 1996.”).

studies selected for inclusion in IARC’s carcinogenicity monograph for glyphosate.<sup>230</sup> Similarly, the studies EPA relied on to conclude that 2,4-D is not carcinogenic are now two decades old, and there is no indication EPA has taken into account the post-1996 studies included in IARC’s carcinogenicity monograph for 2,4-D.<sup>231</sup> EPA may not lawfully ignore more than two decades of scientific and medical research on cancer risk when approving a new pesticide.

By refusing to consider significant new evidence of Enlist Duo’s health risks in general, and cancer and dietary risk in particular, before registering Enlist Duo, EPA lacks an adequate basis to conclude that Enlist Duo would not cause “unreasonable adverse effects” on human health. 7 U.S.C. § 136a(c)(5)(C), (D).

**ii. EPA must correct the flaws in its human health risk assessment for 2,4-D**

As discussed, there are multiple flaws in EPA’s human health risk assessments for 2,4-D in addition to the agency’s failure to consider the current science on 2,4-D’s cancer risk.<sup>232</sup> These flaws prevent EPA from understanding the true extent of the human health risks posed by 2,4-D, which, in turn, prevents the agency from making an accurate determination as to whether registration of Enlist Duo would cause “unreasonable adverse effects” on human health. 7 U.S.C. § 136a(c)(5)(C), (D). EPA must revise its human health risk assessment to correct these flaws prior to making a final registration decision for Enlist Duo.

Even if EPA disagrees with NRDC regarding the extent to which Enlist Duo threatens human health, it is obligated to undertake a scientifically sound assessment of the herbicide’s

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<sup>230</sup> See IARC Glyphosate Monograph, *supra* note 143, 79-92.

<sup>231</sup> See IARC 2,4-D Monograph, *supra* note 14, at 108-26.

<sup>232</sup> See *supra* Section III.C.2.



human health risks to support its conclusions. EPA may not rely on a deficient human health risk assessment to support its proposed registration of Enlist Duo.

**E. EPA may not conditionally register Enlist Duo**

EPA’s proposal to issue a conditional registration for Enlist Duo under 7 U.S.C.

§ 136(c)(7) is improper.<sup>233</sup> The Proposed Registration Decision states:

In situations like Enlist Duo where a company submits an application for a new use on a product that contains two or more active ingredients (combination product), and the use being requested for this combination product is currently registered for one or more of the active ingredients, the EPA only assesses the risks and benefits of the active ingredient that does not currently have products registered for that use.

For the other active ingredient(s), the EPA treats the application as if it were a “me-too,” and does not conduct new assessments for the already registered uses.<sup>234</sup>

Enlist Duo does not, however, qualify for a “me-too” registration. As an initial matter, “me-too” registration applies to pesticides as a whole, rather than to individual ingredients—like glyphosate or 2,4-D—within pesticides. *See* 7 U.S.C. § 136(c)(7)(A) (applying when “*the pesticide* and proposed use” are identical or substantially similar to “any currently registered *pesticide* and use thereof” (emphases added)).

FIFRA authorizes EPA to conditionally register a pesticide only if the agency determines both that “(i) the pesticide and proposed use are identical or substantially similar to any currently registered pesticide and use thereof, or differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment,” and that “(ii) approving the

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<sup>233</sup> *See* EPA, Proposed Registration Decision, at 29 (“The EPA believes that the available data and scientific assessments as well as the overall considerations for benefits for weed management in these important crops support a FIFRA 3(c)(7) registration finding for the proposed uses.”); *id.* at 2 & n.2.

<sup>234</sup> EPA, Proposed Registration Decision, at 2.

registration or amendment in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment.” 7 U.S.C.

§ 136(c)(7)(A). Neither of these conditions is satisfied here.

EPA is not “identical or substantially similar to *any currently registered pesticide* and use thereof,” *id.* (emphasis added). There is no “currently registered pesticide” that combines the active ingredients glyphosate and 2,4-D. This novel combination is unique to Enlist Duo and renders the herbicide substantially dissimilar to any currently registered pesticide. EPA’s Pesticide Registration Manual provides examples of what does, and does not, qualify as identical/substantially similar new pesticide products; Enlist Duo falls squarely within the group of products that do not qualify.<sup>235</sup> For example, the Manual expressly states that “[a]pplications that require review of acute toxicity and efficacy data do not qualify as identical/substantially similar new product applications since submission of such data would indicate that the product is not identical or substantially similar in composition and labeling to the currently registered pesticide identified in the application.”<sup>236</sup> EPA’s analysis of both acute toxicity data and efficacy data (as well as many other additional types of data) in the course of considering whether to register Enlist Duo,<sup>237</sup> is a clear sign that Enlist Duo is not identical or substantially similar to

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<sup>235</sup> See EPA, Pesticide Registration Manual – Chapter 2, *available at* <https://www.epa.gov/pesticide-registration/pesticide-registration-manual-chapter-2-registering-pesticide-product#meetoo> (last visited Dec. 1, 2016).

<sup>236</sup> *Id.*

<sup>237</sup> See, e.g., Kelly M. Lowe, EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide Tolerant Cotton (Oct. 27, 2016), *available at* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0594-0009>; Caleb Hawkins, EPA, Review of Benefits as Described by the Registrant of Enlist Duo 2,4-D choline on Herbicide Resistant Enlist Cotton to Improve the Performance of Current Weed Control Systems and Provide New Weed Resistance Management Options (Oct. 28, 2016), *available at* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0594-0010>; Alexandra McLay,

any currently registered pesticide. In contrast, an example of when a proposed pesticide *is* identical/substantially similar to a currently registered pesticide occurs when an “[a]pplicant is repackaging a registered product that does not require the submission of data nor the submission of a data matrix.”<sup>238</sup>

In addition, Enlist Duo “differ[s] . . . in ways” from “any currently registered pesticide” so as to “significantly increase the risk of unreasonable adverse effects on the environment,” *id.* Compared to any existing glyphosate-based pesticide, Enlist Duo has the additional active ingredient 2,4-D. Particularly taking into account the two- to six-fold increase in use of 2,4-D that is projected to follow registration of Enlist Duo, even the 2,4-D component of Enlist Duo alone substantially increases risks to monarchs and human health. In addition, the combination of 2,4-D and glyphosate will perpetuate high levels of glyphosate use that would otherwise decline, insofar as herbicides that rely on glyphosate alone are losing efficacy. By facilitating increased use of 2,4-D and sustaining (and possibly expanding) glyphosate use—and thereby magnifying the serious risks to monarchs and human health associated with each of these chemicals—Enlist Duo significantly increases the risk of unreasonable adverse effects to the environment beyond the risk posed by any currently registered glyphosate-based pesticide.

Similarly, compared to any existing 2,4-D-based pesticide, Enlist Duo differs in ways that significantly increases the risk of unreasonable adverse effects to the environment. It contains glyphosate, a chemical that poses its own serious risks to monarchs and human health,

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EPA, Human Health Risk Assessment for a Proposed Use of 2,4-D Choline on Herbicide-Tolerant Corn and Soybean (Aug. 8, 2013), *available at* <https://www.regulations.gov/document?D=EPA-HQ-OPP-2014-0195-0007>; Phillips, *supra* note 19.

<sup>238</sup> EPA, *supra* note 235.

and whose use in conjunction with 2,4-D will allow for vastly more of the chemical to be used than would otherwise occur, given the declining efficacy of glyphosate when used alone as an active ingredient. Enlist Duo also extends the use of 2,4-D for the first time to 2,4-D-resistant corn, soy, and cotton (even EPA admits that Enlist Duo will entail use of 2,4-D in new and expanded ways<sup>239</sup>) which, in and of itself, dramatically expands the risks to monarchs and human health discussed in these comments. There is thus no reasonable way to conclude that Enlist Duo “differ[s]” from “any currently registered pesticide” “only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment,” *id.*

EPA misconstrues FIFRA when it claims to have “the authority to issue conditional registrations for pesticide products that are identical or substantially similar in their uses and formulation *to one or more products or for a combination of previously approved products that are already registered and marketed in the United States.*”<sup>240</sup> This interpretation diverges materially from FIFRA, which plainly states that a proposed “me-too” pesticide must be assessed against “any currently registered pesticide,” 7 U.S.C. § 136a(c)(7), and not against a “combination of previously approved products.”

To the extent that EPA is exclusively relying on 40 C.F.R. § 152.113(a), which states that EPA “may [conditionally] approve an application for registration or amended registration of a pesticide product, each of whose active ingredients is contained in one or more other registered pesticide products,” the agency is ignoring other statutory and regulatory requirements for conditional registration. Again, FIFRA provides that a proposed “me-too” pesticide must be identical or substantially similar to “any currently registered pesticide,” 7 U.S.C.

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<sup>239</sup> See EPA, Proposed Registration Decision, at 2, 27.

<sup>240</sup> *Id.* at 2 n.2.

§ 136a(c)(7)(A); it does not suffice that each individual component of a proposed “me-too” pesticide can be found in some combination of currently registered pesticides. 40 C.F.R.

§ 152.113(b) reiterates the requirement that a proposed “me-too” pesticide must be identically or substantially similar to “a currently registered pesticide,” and EPA cannot simply ignore that latter regulatory subsection.

In any event, conditional registration is inappropriate here, because approving Enlist Duo would “significantly increase the risk of . . . unreasonable adverse effect[s] on the environment,” *id.* EPA itself acknowledges that approval of Enlist Duo would greatly expand use of 2,4-D. And, as discussed in these comments, 2,4-D may pose serious risks to both monarchs and human health. In addition, the combination of 2,4-D and glyphosate will perpetuate use of glyphosate at high levels—levels that would otherwise decline given increasing weed resistance to glyphosate alone. Indeed, Enlist Duo is specifically designed to overcome the weed resistance problems that would limit the use of glyphosate alone.<sup>241</sup> Approval of Enlist Duo would thus also significantly increase risks to both monarchs and humans from glyphosate. Even if EPA believes that approval of Enlist Duo would have no effect on overall levels of glyphosate use, the agency cannot so conclude without conducting an actual assessment of how registration of Enlist Duo may affect overall use of glyphosate. Without such an assessment, EPA cannot reasonably make a finding that registration of Enlist Duo would not “significantly increase the risk of any unreasonable adverse effect on the environment,” 7 U.S.C. § 136a(c)(7).

In sum, contrary to the requirements of 7 U.S.C. § 136a(c)(7)(A), Enlist Duo is *not* “substantially similar to any currently registered pesticide,” it does *not* “differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment,”

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<sup>241</sup> See, e.g., EPA, Proposed Registration Decision, at 27.

and approving the registration in the manner proposed *would* “significantly increase the risk of any unreasonable adverse effect on the environment.” It would thus be unlawful for EPA to conditional register Enlist Duo.

#### IV. CONCLUSION

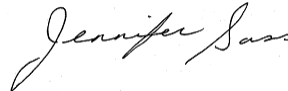
Enlist Duo poses serious risks to both human health and monarch butterflies. EPA must adequately consider these risks before making a final registration decision for the herbicide.

Respectfully submitted,



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# SPRING RECOLONIZATION OF EASTERN NORTH AMERICA BY THE MONARCH BUTTERFLY: SUCCESSIVE BROOD OR SINGLE SWEEP MIGRATION?

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**ABSTRACT.** Each March, monarch butterflies, *Danaus plexippus* (L.), remigrate north from Mexican overwintering sites. By June they are distributed across most of eastern North America, north to southern Canada. Here we distinguish between two alternative spring recolonization strategies: (1) "single sweep recolonization," in which overwintered individual remigrants from Mexico were hypothesized to colonize the entire eastern North American breeding range, and (2) "successive brood recolonization" in which remigrants from Mexico were hypothesized to lay all of their eggs in the southern United States and then die, leaving their offspring to continue the migration northward and recolonize the northern breeding range.

Cardenolide concentrations, cardenolide "fingerprints," and wing wear of migrant butterflies captured along two latitudinal transects in the spring of 1985 provided three lines of independent support consistent with hypothesis 2. Monarchs captured in the southern United States in late March and early April, which lay eggs on the emergent milkweeds, had (1) low cardenolide concentrations, (2) cardenolide fingerprints identical to those of overwintering monarchs in Mexico, and (3) very worn wings. In contrast, monarchs from the northern United States in May and June had (1) high cardenolide concentrations, (2) fingerprints characteristic of the southern spring milkweed flora, and (3) wings in significantly better condition. Thus, monarchs recolonize their breeding range each spring by migration of successive broods and not by a single sweep of overwintered migrants.

The existence of concurrent migration and reproductive activity of the new spring brood is an exception to the "oogenesis-flight syndrome" of migratory insects, which holds that reproductive activity is suppressed during migration. The new evidence also indicates that chemical defense during the monarch's annual life cycle is dynamic and cycles from poorly protected autumn migrants, which overwinter and then recolonize the southern United

States, to highly protected migrants of the first spring generation, which recolonize the northern United States.

## INTRODUCTION

The monarch butterfly, *Danaus plexippus* (L.), is perhaps the best-known example of a regular long-range insect migrant. From the long-term research on monarch movement by Urquhart (1960, 1966, 1976, 1987) and Urquhart and Urquhart (1976a,b,c, 1977, 1978; summaries in Brower, 1985; Malcolm, 1987), we know that monarchs that breed east of the Rocky Mountains fly southwest each autumn, from the northern United States and southern Canada to the transvolcanic mountains of central Mexico, where they spend the winter in dense aggregations on high-altitude fir trees (Calvert and Brower, 1986). In spring the same butterflies make the return journey to at least the southern United States, and by mid-summer the species is once again distributed as far north as southern Canada (Urquhart and Urquhart, 1979). The geographical area occupied by monarchs east of the Rocky Mountains (Urquhart, 1960) almost exactly coincides with the distribution of the most common larval host plants in the milkweed genus *Asclepias* (Fig. 1).

Although the monarch's migration and their larvae feeding on milkweeds are familiar phenomena, little is known of the critical spring migration and the annual distribution of monarch generations in time and space across eastern North America. As can be seen in Williams (1958), Urquhart (1960), and Brower (1961, 1985) two hypotheses have emerged to explain how monarchs migrate to recolonize North American milkweeds each spring. (1) The first, which we now formalize as a "single sweep recolonization strategy," holds that overwintered monarchs remigrate from Mexico to de-



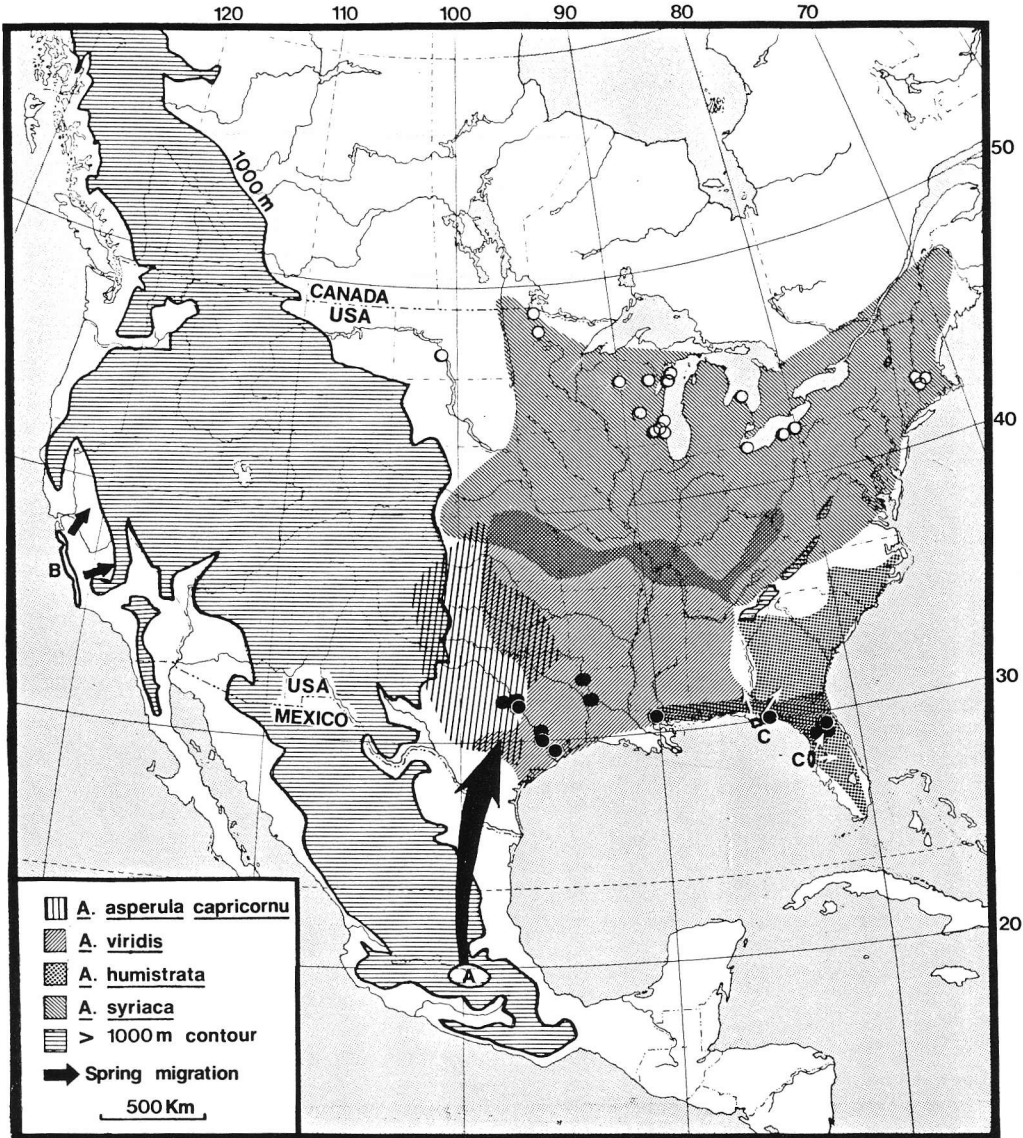
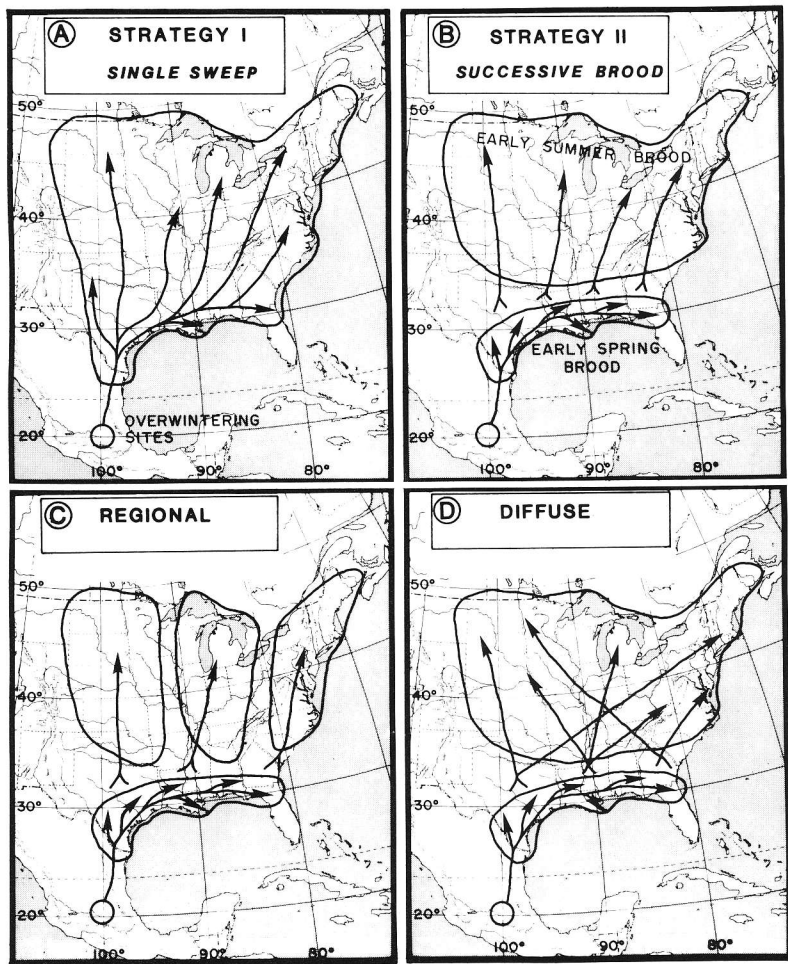


Figure 1. The distribution of the three abundant southern milkweeds, *A. asperula capricornu*, *A. viridis*, and *A. humistrata*, and the most abundant northern milkweed, *A. syriaca* (after Woodson, 1954). The breeding distribution of migratory eastern monarchs, which overwinter at A in Mexico, coincides largely with the distributions of these milkweed species east of the Rocky Mountain barrier. This barrier is indicated by most of the horizontal hatched area within the 1,000-m contour in the western United States and Canada. Monarchs that overwinter at C in Florida may contribute small numbers to the eastern spring migration, whereas monarchs that overwinter at B in California appear largely restricted to migration within California. Our 1985 adult monarch sample collection sites in the eastern United States are also shown (black circles = southern samples, white circles = northern samples).

posit eggs on milkweeds over their entire eastern range (Fig. 2A). It is this purported strategy that has prevailed in the literature (Johnson, 1969; Ackery and Vane-Wright, 1984; Brower, 1985; Urquhart, 1987). (2) Alternatively, monarchs could locate milkweed resources over their entire breeding range by a “successive brood recolonization” strategy (Fig. 2B). Overwintered monarchs would deposit all of

their eggs on early-spring milkweeds in the southern states of the United States and then die, leaving their offspring to migrate north and recolonize the remainder of the northern range. If recolonization is achieved by the second, successive brood strategy, then the new spring generation may migrate “regionally” (Fig. 2C) or “diffusely” (Fig. 2D) across North America.





**Figure 2.** Hypothesized monarch spring migration strategies: (A) “single sweep recolonization”—overwintered migrants migrate north and lay eggs on milkweeds over their entire spring and summer range—(B) “successive brood recolonization”—overwintered migrants lay all of their eggs on southern United States milkweeds, leaving their offspring to recolonize northern milkweeds. (C and D) Two alternative ways in which successive brood recolonization could occur by either “regional” migration, directed north along specific corridors, or “diffuse” migration, directed approximately and randomly north.

Urquhart’s (1960) pioneering mark–recapture technique with monarch butterflies led to the discovery in 1975 of their overwintering location in Mexico (Urquhart, 1976). However, the small numbers of spring migrant individuals recaptured (Malcolm, 1987) are insufficient to differentiate the possible modes of their remigration. Here we solve this problem by utilizing chemical information stored in the butterflies during their larval stage to assess the spatial and temporal origins of each field-collected adult monarch during their spring migration.

Monarch larvae have been recorded feeding on 27 of the 108 North American milkweed species in the genus *Asclepias* (Malcolm and Brower, 1986), and adult butterflies reflect the characteristic chemical pattern or “fingerprint” of different cardenol-

ides (cardiac-active steroidal glycosides and genins) present in the host-plant species fed upon by their larvae (Roeske et al., 1976; Brower et al., 1982, 1984a,b; Cohen, 1985; Seiber et al., 1986; Lynch and Martin, 1987, this volume; Martin and Lynch, 1988; Malcolm et al., 1989; Nelson, this volume). Since Woodson (1954) gives extensive information on the distribution of *Asclepias* species, the origin of a monarch can be assigned to each milkweed’s distribution, samples of which are shown in Figure 1. Furthermore, the common southern milkweeds *Asclepias humistrata*, *Asclepias viridis*, and *Asclepias asperula capricornu* have considerably higher cardenolide concentrations than the extremely abundant northern *Asclepias syriaca* (Table 1). Monarchs reared from these milkweeds reflect these

cardenolide concentrations (Table 1), so that individuals originating in the southern United States from southern milkweeds can be expected to have higher cardenolide concentrations than those that fed on milkweeds in the northern United States and Canada. The trend for lower cardenolide contents of northern, *A. syriaca*-derived butterflies is even more pronounced in the March and April remigrants from Mexico because these monarchs lose cardenolide during migration and the overwintering period (Malcolm and Brower, 1989; Malcolm et al., 1989).

In the spring of 1985 we collected monarchs along an early spring, southern latitudinal transect in April and a later, northern latitudinal transect in May and June. We collected the first arrivals of migrating monarchs at these latitudes across the eastern United States, from Texas and Louisiana to Florida in the south, and North Dakota, Minnesota, Wisconsin, Michigan, Ohio, Pennsylvania, and Massachusetts in the north. As measures of monarch host-plant origin, age, activity, and distance moved, we determined the cardenolide concentrations, cardenolide fingerprints, and wing wear of these butterflies.

## METHODS

The cardenolide concentrations of ether-defatted monarchs were measured by spectrophotometry at an absorbance wavelength of 622 nm on a Perkin-Elmer 559A spectrophotometer (Brower et al., 1982; Malcolm et al., 1989). Cardenolide concentrations were determined as  $\mu\text{g}$  cardenolide/0.1 g dry, defatted weight of butterfly, to control for variation in the fat weights carried by monarchs at different stages in their life history (Brower, 1985). Cardenolide fingerprints were produced by thin-layer chromatographic (TLC) separation of cardenolides in these extracts, after pigments were precipitated with lead acetate (Brower et al., 1975, 1982, 1984a; Malcolm et al., 1989). Silica gel 60 F<sub>254</sub> TLC plates were run four times in chloroform:methanol:formamide (90:6:1, v:v) at 20°C and cardenolides were visualized as blue spots by spraying with a saturated solution of 2,2'-4,4'-tetranitrodiphenyl in benzene, followed by 10% potassium hydroxide in 50% aqueous methanol (Brower et al., 1982).

To determine the identity of cardenolide fingerprints in wild-caught monarchs, we compared their TLC cardenolide patterns with those of monarchs reared from a selection of known milkweeds. At present our comparative library of fingerprints includes monarchs reared from the *Asclepias* species that occur commonly in various parts of North America, east of the Rocky Mountains—*humistrata*, *viridis*, *curassavica*, *exaltata*, *speciosa*, and *syriaca* (see Fig. 3 for TLC examples). The fingerprints of monarchs reared on *A. viridis* and *A. asperula capricornu* in Texas and Louisiana have also been determined by Lynch and Martin (1987) and Martin and Lynch (1988). Since the cardenolide fingerprints of spring migrant monarchs were remarkably consistent, with almost all butterflies showing one of two very distinct patterns, we used published accounts of the predominant cardenolides to

establish the identity of fingerprints as objectively as possible. The criteria we used for fingerprint identification included comparison of spot intensities and spot mobilities (relative to digitoxin) with published values. For example, the fingerprint of butterflies that fed as larvae on *A. syriaca* is dominated by the cardenolide glycoside aspecioside—the largest and most intense spot that appears approximately halfway between the origin and digitoxin in Figure 3 (see also Malcolm et al., 1989). The only other known fingerprint like this is for monarchs reared from *Asclepias speciosa* (Brower et al., 1984a; Seiber et al., 1986), but because this is a western equivalent of *A. syriaca* we think it is of only marginal significance to monarch breeding east of the Rockies. The other dominant fingerprint in our samples is derived from *A. viridis* and is characterized by more lower polarity cardenolides that move farther up the TLC plate than the cardenolides in *A. syriaca* monarchs. The two obvious sets of paired spots on either side of digitoxin (Fig. 3) are characteristic of *A. viridis*-derived monarchs. The small number of additional fingerprints were always very obviously different from the two predominant patterns. Although our fingerprint identifications do include a degree of subjective pattern comparison we consider that the fingerprints were so consistent within samples that our method is robust (see also Seiber et al., 1986). However, we are developing densitometry measurements of TLC patterns followed by discriminant analyses among sample patterns (Cockrell, Malcolm, and Brower, in prep.).

The location, date, and number of monarchs collected across the two transects, in the spring 1985 samples, are given in Table 2. The collections were designed to sample monarchs east of longitude 105°W in the United States, with western (<35°N, >90°W) and eastern (<35°N, <90°W) divisions of the southern range, and western (>40°N, >80°W) and eastern (>40°N, <80°W) divisions of the northern range (see Fig. 1). Thus, our southern samples are from Texas, Louisiana, and Florida, and the northern samples are from North Dakota east to western Pennsylvania, and Massachusetts (Table 2). The small samples of 19 and 13 from the southeast and northeast in comparison with more western samples of 114 and 633 reflect the much lower numbers of monarchs during the spring in the east. The dates of collection (Table 2) coincided with the known times of arrival of migrant monarchs; late March and early April in the Gulf states and late May and early June in Wisconsin and Minnesota (Brower, 1985; Malcolm et al., 1987; Cockrell et al., this volume; Lynch and Martin, this volume; Riley, this volume).

Cardenolide concentrations were determined from 562 Mexican overwintering monarchs collected in January, February, and March 1978 and 1979. We chromatographed 386 of these butterflies (Table 2). These samples were considered representative of typical overwintering cardenolide contents because Malcolm and Brower (1989) have shown that the cardenolide concentrations and amounts in eight samples, representing 839 monarchs from four overwintering seasons in Mexico, were all extremely similar.

Field-collected monarchs were stored frozen in glassine envelopes. In the laboratory, spermatophores were dis-



Table 1. Cardenolide concentrations in the leaves ( $\mu\text{g}/0.1\text{ g}$  dry weight) of three of the most abundant southern and the single most abundant northern *Asclepias* species, with the cardenolide concentrations of monarch butterflies reared on these four milkweed species.

<i>Asclepias</i> species	Origin	N <sup>a</sup>	Plant			Butterfly		
			Mean	SD	Range	Mean	SD	Range
Southern								
<i>asperula</i> <sup>b</sup>	TX	41	886	255	341–1,616	363	77	231–515
<i>viridis</i> <sup>c</sup>	LA	60	245	70	95–432	337	105	73–591
<i>viridis</i> <sup>d</sup>	FL	18	376	203	148–972	438	63	337–548
<i>humistrata</i> <sup>d</sup>	FL	22	389	141	71–639	337	65	242–478
Northern								
<i>syriaca</i> <sup>e</sup>	— <sup>e</sup>	158	50	39	4–229	234	150	0–792

<sup>a</sup> Number of plant–butterfly paired samples.  
<sup>b</sup> Martin and Lynch (1988).  
<sup>c</sup> Lynch and Martin (1987).  
<sup>d</sup> S.B. Malcolm, material collected near Gainesville, Florida, 1983 and 1984.  
<sup>e</sup> Malcolm et al. (1989); North Dakota east to Vermont and south to Virginia (see Fig. 1).

sected from the bursa copulatrix of females to eliminate possible male contribution to female cardenolides. Overwintering butterflies were not dissected since few females were mated (Brower, 1985; Van Hook, this volume). Wing wear of all butterflies was estimated by the same person on a subjective 0.5 interval scale of 1 (perfect condition) to 5 (extremely worn) based on degree of scale loss, wing fading, and extent of tearing and fraying. All butterflies were photographed to maintain a consistent wing wear estimate and reference record. Butterflies were then dried at 60°C for 16 hr, before fat extraction and cardenolide analysis.

RESULTS

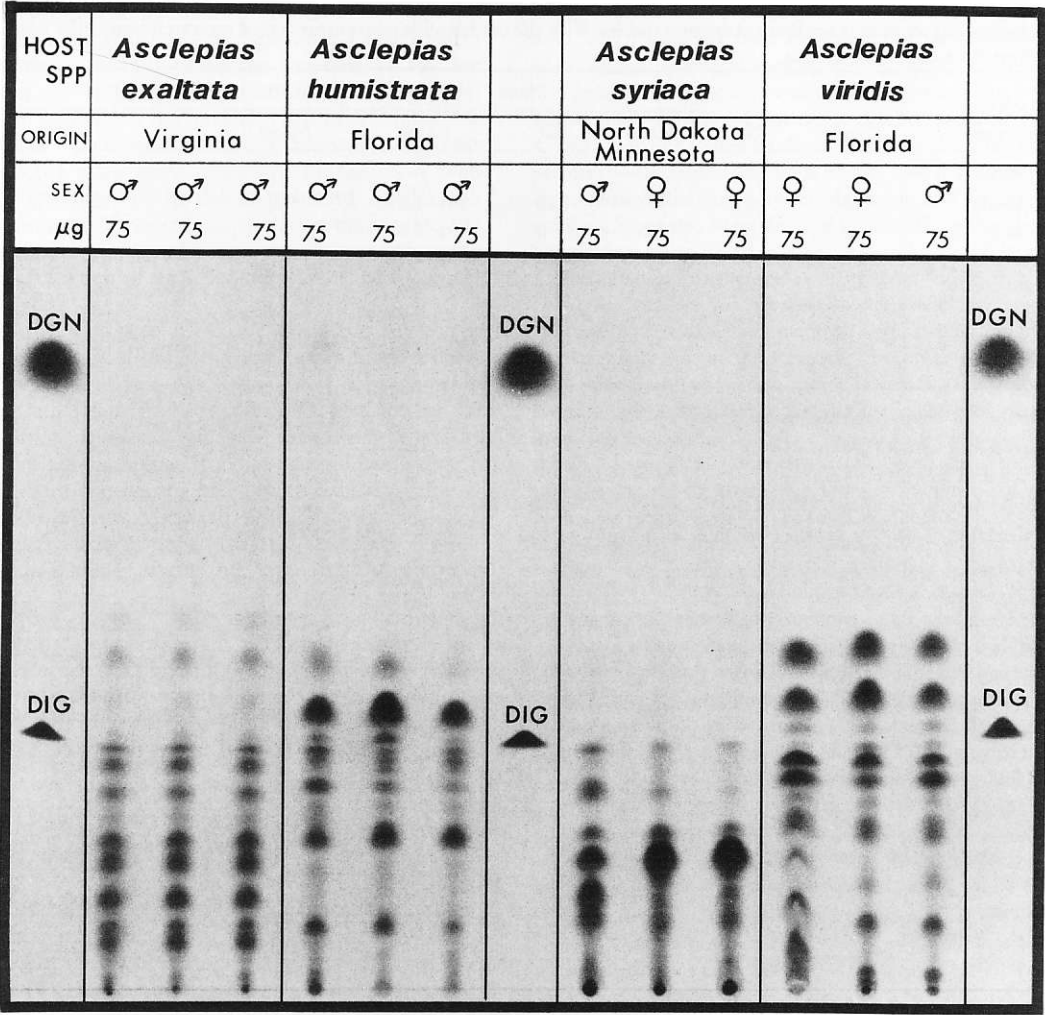
CARDENOLIDE CONCENTRATIONS

Although the five samples of overwintering monarchs from Mexico (Table 2) showed variation among their mean cardenolide concentrations (Kruskal-Wallis ANOVA,  $H_4 = 17.4$ ;  $p < 0.01$ ), only sample 2 (Sierra Chincua, 23 January 1979) had significantly less cardenolide (mean = 63  $\mu\text{g}/0.1\text{ g}$ ) than samples 1 and 3 (means = 97 and 91  $\mu\text{g}/0.1\text{ g}$ , respectively; Student-Newman-Keuls range test at 5%). Samples 4 and 5 (means = 81 and 72  $\mu\text{g}/0.1\text{ g}$ , respectively) were not significantly different from any of the other samples. Since sample 2 was the only 1979 sample and the means of all samples were similarly low (between 50 and 100  $\mu\text{g}$ ), the differences were considered sufficiently small to permit pooling of the overwintering samples for comparison with wild-caught migrant monarchs.

The mean cardenolide concentrations in monarchs from overwintering sites in Mexico (77  $\mu\text{g}/0.1\text{ g}$ ) and from the early-spring samples in Florida, Louisiana, and Texas in the southern United States (62  $\mu\text{g}/0.1\text{ g}$ ) were not significantly different (Table 3; the 1979 Mexican sample described above makes no difference to the significance of this result), and

the Mexican and southern United States groups show extremely similar distributions strongly skewed to the right (Fig. 4a). In contrast, the northern United States monarchs show a broad, normal distribution of cardenolide concentrations (Fig. 4b) and, on average, contained more than twice the amount of cardenolide (157  $\mu\text{g}/0.1\text{ g}$ ; Table 3). The highly significant difference between the Mexican and southern versus the northern data sets is strong evidence that the spring migrants arriving at the southern United States in early April are from the same population of monarchs that leave Mexico in mid- to late March, whereas the northern population fed as larvae on southern milkweeds, which are rich in cardenolides (Lynch and Martin, 1987, this volume; Martin and Lynch, 1988). We conclude that monarchs arriving in the northern United States are the first-generation offspring of the overwintered spring remigrants, i.e. that the successive brood recolonization strategy prevails.

Although the sample sizes for the eastern monarchs were small compared with those for the more western samples, the skewed southern and normal northern patterns of low to high cardenolides remained similar (Fig. 5a and 5b). However, the southern monarchs in the east had significantly higher cardenolide concentrations than the more western ones (Table 4). Most of this difference was caused by three monarchs with >200  $\mu\text{g}/0.1\text{ g}$  of cardenolide. These individuals may have fed as larvae on the high cardenolide milkweed *Asclepias curassavica* in south Florida (Cohen, 1985; Malcolm and Brower, 1986), or even on the high cardenolide *Asclepias nivea* from Caribbean islands (Roeske et al., 1976). Alternatively, they may have fed on *A. humistrata* in Florida, but their early presence in north Florida in mid-April required either unusually early larval development or remarkable longevity, because *A. humistrata* is only avail-



**Figure 3.** TLC fingerprints of larval host-derived cardenolide spots in adult monarchs reared from the milkweeds *A. exaltata*, *A. humistrata*, *A. syriaca*, and *A. viridis*. Each of the 12 monarch extracts were applied to the plate so that equal quantities of cardenolide per butterfly (75 μg) were separated during plate development. The cardenolide standards digitoxin (DIG) and digitoxigenin (DGN) are also shown.

able from early March until mid-July (Malcolm et al., 1987). In contrast, the high cardenolide concentrations of northeastern monarchs were not significantly different from those of the more western samples (Table 4).

CARDENOLIDE FINGERPRINTS

The most striking initial result of examining the TLC patterns of wild-caught monarchs is the small number of different cardenolide fingerprints that we found. In 94% of 1,148 wild overwintering and spring migrant monarchs we identified three common patterns (Table 5). The remaining 6% had zero or indeterminate cardenolide that could not be assigned a fingerprint (Table 5). Of these fingerprints,

92% Mexican and 84% southern United States monarchs had fingerprints that were assignable to *A. syriaca* [Table 5 and Fig. 6, compare the *A. syriaca* pattern of Malcolm et al. (1989), which is also illustrated in Fig. 3, with the Mexican and southern United States monarch patterns]. This fingerprint is easily recognizable by the high polarity of the most concentrated cardenolides such as aspecioside, syriocide, and syriobioside (Seiber et al., 1986; Malcolm et al., 1989). Thus almost all of the overwintered, Mexican butterflies that remigrate to the southern United States in spring must have fed as larvae on *A. syriaca* the previous year in the northern United States (contingency table comparison of fingerprint frequencies,  $\chi^2 = 0.3$ ,  $df = 2$ , NS). Of the remaining southern monarchs, 9% had



Table 2. The location, date, and numbers of overwintering and migrating monarch adults [males (M), females (F), and totals] collected for analyses of cardenolide concentration, cardenolide fingerprint, and wing wear (see Fig. 1 for the distribution of collection sites).

Location	Date	Sample size		
		M	F	Total
Overwintering monarchs in Mexico <sup>a</sup>				
1. Sierra Chincua, Michoacán	15 January 1978	58	47	105 (83) <sup>b</sup>
2. Sierra Chincua, Michoacán	23 January 1979	79	77	156 (97)
3. Cerro Pelón, México	5 February 1978	50	50	100 (80)
4. Cerro Altamirano, Michoacán	15 February 1978	50	50	100 (70)
5. Sierra Chincua, Michoacán	20 March 1978	51	50	101 (56)
Totals		288	274	562 (386)
Migrating monarchs in the United States (all 1985)				
Southern samples				
1. Central Texas (Counties: Travis, Gonzales, Blanco, De Witt, Refugio)	5–25 April	28	17	45
2. West Louisiana (Counties: Natchitoches, Caddo/DeSoto, St. Tammany)	6–28 April	37	24	61
3. Central Texas (County: Blanco)	9–12 May	5	3	8
4. North-central Florida (Counties: Franklin, Alachua, Levy, Putnam)	30 March–5 May	6	13	19
Totals		76	57	133
Northern samples				
1. East Wisconsin (Counties: Ozaukee, Milwaukee, Waukesha)	22–31 May	31	15	46
2. West Pennsylvania (County: Erie)	30 May	4	3	7
3. North Ohio (Counties: Ashtabula, Ottawa)	31 May–1 June	10	3	13
4. East Wisconsin (Counties: Ozaukee, Milwaukee, Door, Langlade, Taylor, Marquette, Waukesha)	1–9 June	221	80	301
5. East Michigan (County: Huron)	2–3 June	56	23	79
6. Central North Dakota (County: Oliver)	14–18 June	15	4	19
7. North Minnesota (County: Beltrami)	19–20 June	42	30	72
8. East Wisconsin (County: Door)	24 June	62	34	96
9. Central Massachusetts (Counties: Hampshire, Franklin)	26 May–22 June	8	5	13
Totals		449	197	646

<sup>a</sup> See Calvert and Brower (1986) for details of overwintering locations in Mexico.

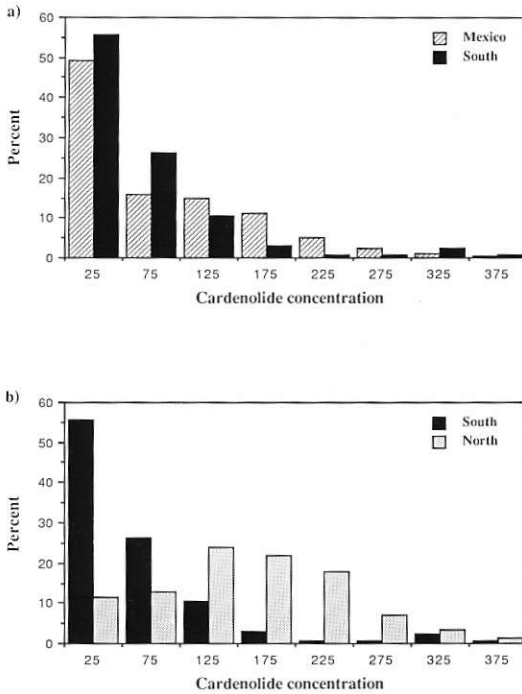
<sup>b</sup> Mexican samples in parentheses were used for TLC analyses; otherwise TLC analyses were performed on all monarchs in a sample.

insufficient cardenolide to establish a fingerprint, and the other 7% may have been collected sufficiently late to be first-generation monarchs that fed as larvae on *A. viridis* and *A. humistrata* (Table 5). In marked contrast, 84% of northern monarchs had a fingerprint characteristic of the southern milk-

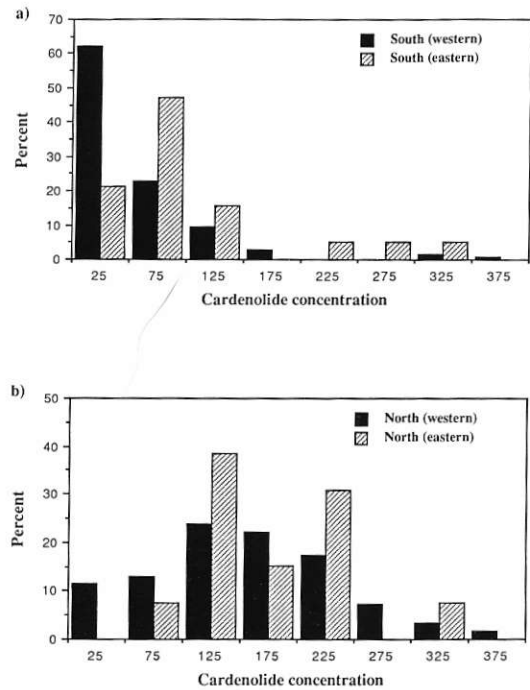
weed *A. viridis*, and 6% had the southeastern *A. humistrata* fingerprint (Table 5 and Fig. 6, compare the *A. viridis* pattern of Figure 3 with the northern United States monarch pattern). Thus, northern monarchs had very different fingerprints from those of southern monarchs (contingency table  $\chi^2 = 471.3$ ,

Table 3. Comparisons of the cardenolide concentrations ( $\mu\text{g}$  cardenolide/0.1 g dry lean weight) in Mexican overwintering monarchs (Mexico), southern spring migrants (South), and northern spring migrants (North). Samples are compared by either Mann-Whitney *U* or Wilcoxon two-sample tests with significance set at 5%.

Monarch sample	N	Cardenolide concentration			Test statistic	
		Mean	SD	Range	<i>z</i>	<i>p</i>
Mexico	562	79	80	0–366	1.00	>0.16 NS
South	133	62	65	0–354		
North	646	157	81	0–395		



**Figure 4.** Comparisons of the distributions of cardenolide concentrations in (a) Mexican overwintering monarchs (Mexico) and southern United States monarchs (South) and (b) southern (South) and northern (North) United States monarchs. See Table 2 for sizes, dates, and locations of samples, and Table 3 for cardenolide concentration data.



**Figure 5.** Regional comparisons of the cardenolide concentration distributions in (a) western and eastern samples of southern United States monarchs and (b) western and eastern samples of northern United States monarchs. Locations and times of collection of these monarchs are given in Table 2.

$df = 3$ ,  $p = 0.0001$ ) and most of them must have reached their northern range by a successive brood recolonization strategy, dependent on the abundance of *A. viridis* throughout the southern states—especially Texas. The 6% of *A. syriaca* monarchs in the northern samples may represent either new, first spring generation butterflies reared from *A. syriaca* or overwintered migrants. If these were overwintered migrants, then a small percentage of monarchs may have completed the spring recolonization of their breeding range by a single sweep strategy.

It is interesting to note that although the north-eastern sample was small (Table 6) significantly more *A. humistrata* fingerprints (67%) and significantly fewer *A. viridis* fingerprints (17%) were detected than expected in these monarchs, than in north-western monarchs (contingency table  $\chi^2 = 45.8$ ,  $df = 3$ ,  $p = 0.0001$ ). Consequently, the converse was also significant in that more *viridis*-derived, and fewer *humistrata*-derived monarchs occurred than expected in more western samples from the north. This regionality suggests that the first-generation spring migrants that fed as larvae on *A. humistrata* in the southeastern coastal plain were largely constrained to the eastern side of the Appalachian

mountains in their northward migration, whereas west of the Appalachians monarchs are likely to be derived from *A. viridis*.

Each cardenolide fingerprint of the three south-eastern, high cardenolide monarchs described above is most like that derived from *A. humistrata*. Thus, despite their early arrival in Florida (9, 15, and 17 April), we think these monarchs may have been very early first-generation butterflies that fed as larvae on *A. humistrata* in south Florida during March (see Cockrell et al., this volume, for details of breeding constraints in Florida). This may indicate the significance of monarchs overwintering in Florida (Brower, 1985) for early recolonization of south-eastern milkweeds.

## WING WEAR

The condition of monarch wings is consistent with the cardenolide evidence for successive brood recolonization of North American milkweeds. Monarchs collected in the southern United States in April had wings in significantly worse condition than monarchs from the northern United States in May and June (Fig. 7 and Table 7), suggesting that later, northern arrivals were younger migrants than earlier, southern arrivals.

In regional comparisons, the wings of south-



Table 4. Comparisons of the cardenolide concentrations ( $\mu\text{g}$  cardenolide/0.1 g dry lean weight) in spring migrant monarchs from western and eastern subsamples of butterflies collected in the south and north of the United States east of the Rocky Mountains. Samples were compared by Wilcoxon two-sample tests with significance at 5%.

Monarch sample	N	Cardenolide concentration			Test statistic	
		Mean	SD	Range	z	p
South						
Western	114	56	60	0–354	2.84	0.005
Eastern	19	99	82	12–314		
North						
Western	633	156	81	0–395	1.04	0.30 NS
Eastern	13	177	65	57–305		

western and southeastern monarchs (Fig. 8a) showed no significant difference in wing wear (Table 8). Despite the overall, similarly worn wings of eastern and more western monarchs from the south, the three southeastern monarchs with the high cardenolide concentrations described above also had wings in good condition with scores of 1.5, 2, and 2.5. This suggests that these butterflies were not overwintered migrants and that they had not flown very far, or for very long, before being caught in north-central Florida.

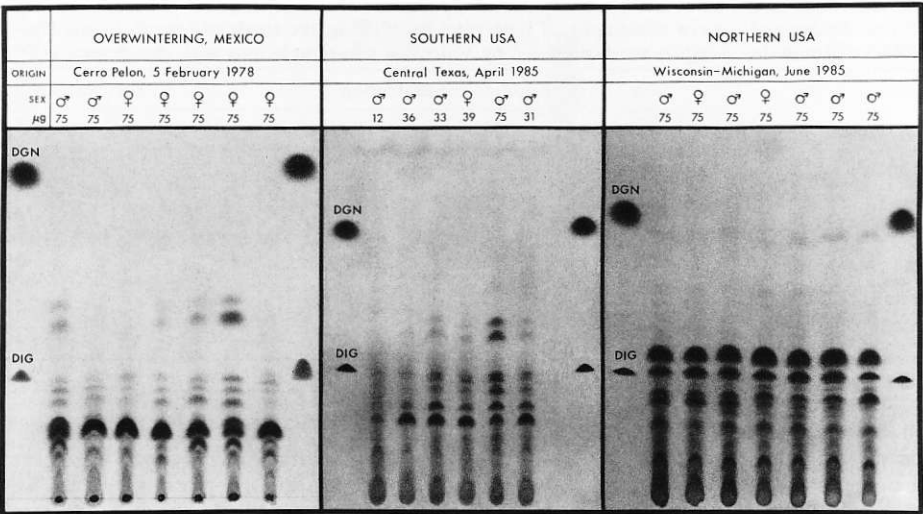
In contrast, the wings of northwestern monarchs were significantly more worn than those of northeastern monarchs (Fig. 8b and Table 8). The small sample of northeastern monarchs were all in excellent condition, whereas the large northwestern sample of 646 butterflies included 46 battered monarchs, with wing wear scores of 4, 4.5, and 5, caught in June (Fig. 8b). Since 43 of these butterflies showed the *A. viridis* fingerprint and 2 had the *A. humistrata* fingerprint (with one unknown), and 43 of the 46 were late June arrivals, caught on either 20 or 24 June in Minnesota and Wisconsin (3 were caught on 6, 8, and 9 June in Wisconsin), these must have been first-generation monarchs from farther south that had sufficient time to become well worn during migratory and reproductive activities. Interestingly, 80% of these 46, mostly late monarchs were females, which was a significantly higher percentage than the 30% of females in the complete sample of 646 northern butterflies ( $\chi^2 = 45.6$ ,  $df = 1$ ,  $p = 0.0001$ ; Table 2). There were also significantly more females in this worn subsample than in the total sample of 162 late monarchs caught on 20 and 24 June ( $\chi^2 = 21.1$ ,  $df = 1$ ,  $p = 0.0001$ ). Thus, the worn females provide good evidence for the “costs” of activities such as mating and egg laying. In contrast, the male-biased sex ratio of the 162 late, less-worn monarchs did not differ from the total northern sample of 646 monarchs ( $\chi^2 = 3.2$ ,  $df = 1$ ,  $p = \text{NS}$ ).

On first consideration, these worn butterflies suggest the presence of overwintered migrants that used a single sweep recolonization strategy to reach their northern breeding area. However, their remarkably consistent *A. viridis* fingerprint dispels this sugges-

tion. A further group of butterflies, the 6% of northern arrivals with the *A. syriaca* fingerprint (Table 5), could also support the single sweep hypothesis if they showed well-worn wings characteristic of old and overwintered long-distance migrants. However, unlike the group of well-worn butterflies, these 35 *A. syriaca*-derived butterflies had the same sex ratio as the total northern sample ( $\chi^2 = 1.3$ ,  $df = 1$ ,  $p = \text{NS}$ ) and had wings in relatively good condition with a mean score of 2.4 ( $SD = 0.5$ ), which was almost identical to that of the total northern sample (Table 7). Most of these *A. syriaca*-derived monarchs were collected in Minnesota, Wisconsin, and Michigan (plus one from Ohio and one from Massachusetts) between 29 May and 9 June (two were collected on 20 June and two on 24 June). Because of their good condition, we conclude that they too are first spring generation individuals which must have exploited early flushing *A. syriaca* in May as the plants first appeared at the southern edge of this milkweed’s distribution, from Kansas east to Kentucky (Fig. 1).

Table 5. Percentages of the different *Asclepias* species-specific cardenolide fingerprints assigned by TLC in 1,148 Mexican overwintering monarchs and spring migrant monarchs from the southern and northern United States. Three fingerprint patterns were recognized in 94% of the total samples, and 6% of the total was indeterminate.

<i>Asclepias</i> species	Migrants		
	Over-wintering Mexico (%)	Southern United States (early spring)	Northern United States (late spring)
		(%)	(%)
<i>viridis</i>	0	3	84
<i>humistrata</i>	0	4	6
<i>syriaca</i>	92	84	6
Indeterminate	8	9	5
Sample size	386	133	629



**Figure 6.** Composite TLC plate of cardenolide fingerprints in monarchs from a Mexican overwintering site (Cerro Pelón), the southern United States (central Texas), and the northern United States (Wisconsin and Michigan). Mexican and southern monarchs have the *A. syriaca* fingerprint shown in Figure 3, and the northern monarchs show the *A. viridis* fingerprint. Where possible each sample was applied to the plate so that equal quantities of cardenolide per butterfly (75 µg) were separated in the solvent system. Only one of the southern monarchs contained sufficient cardenolide, since the other five butterflies had the very low cardenolide concentrations and variable spot intensities, characteristic of southern spring remigrants. The three different TLC plates are aligned by the spot origin and by the cardenolide glycoside standard digitoxin (DIG). The cardenolide genin digitoxigenin (DGN) is also applied to each plate, but variation among plates usually makes it impossible to align plates at both standards. However, via chromatography with additional known plant cardenolides we know that most overwintering monarchs and southern spring arrivals have the same fingerprint pattern, derived from the northern milkweed *A. syriaca* (see Malcolm et al., 1989, for details).

DISCUSSION

Our results show distinct differences in the cardenolide concentrations, cardenolide fingerprints, and wing conditions between the first migrant monarch arrivals in the southern and northern United States. All three sets of evidence show that nearly all of the spring migrant arrivals in the north are the offspring of overwintered remigrants that arrived in the southern United States from Mexico. In Texas and Louisiana these overwintered remigrants laid most of their eggs on the extremely abundant early-spring, southern milkweed *A. viridis*. This milkweed has high concentrations of cardenolide, which produces monarchs with correspondingly high cardenolide concentrations. Although these monarchs appear to lose some of this host-derived cardenolide during their northward, spring migration (Malcolm and Brower, 1989) it is these monarchs that reach the northern United States, with very few, if any, of their overwintered parents reaching as far north as Wisconsin or Michigan. Thus, the spring remigration of monarchs, to recolonize their milkweed larval food resources, is achieved almost exclusively by a successive brood strategy and not by a single sweep of overwintered Mexican migrants. Using completely different methods, which focus on the abiotic constraints faced by monarchs

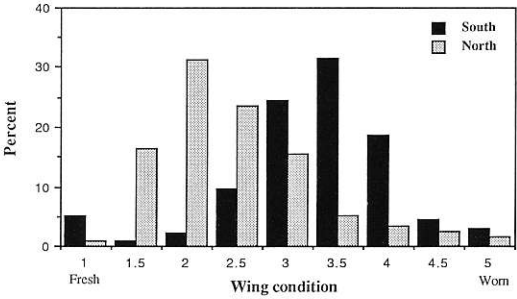
as they migrate north in the spring, Cockrell et al. (this volume) reach the same conclusion.

The monarchs produced in the first spring generation also show some regionality in their continued northward migration and colonization. Thus, those that fed as larvae on the common south-eastern milkweed, *A. humistrata*, are later found as far north as Massachusetts. Similarly *A. viridis* is extremely abundant in Texas and Oklahoma and

**Table 6.** Regional percentages of the different *Asclepias* species-specific cardenolide fingerprints identified by TLC in spring migrant monarchs from the western and eastern subsamples of monarchs from the south and north of the United States, east of the Rocky Mountains.

<i>Asclepias</i> species	South		North	
	West-ern (%)	East-ern (%)	West-ern (%)	East-ern (%)
<i>viridis</i>	4	0	85	17
<i>humistrata</i>	0	26	5	67
<i>syriaca</i>	87	69	5	17
Indeterminate	10	5	5	0
Sample size	114	19	623	6





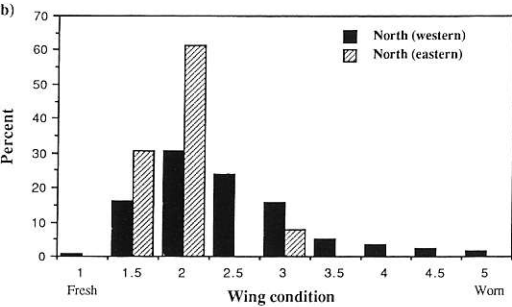
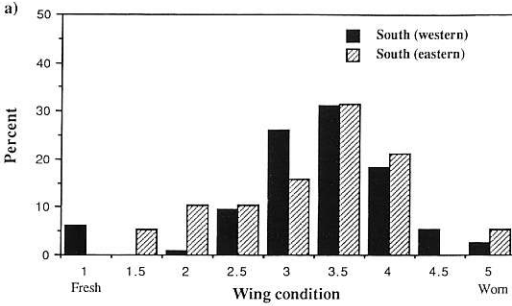
**Figure 7.** Wing wear distributions of monarch spring arrivals in the southern (South) and northern (North) United States (1 = fresh wings in pristine condition, in 0.5 intervals to 5 = extremely worn and tattered wings). The samples are compared statistically in Table 7.

monarchs with the *A. viridis* fingerprint dominate the monarch samples collected in North Dakota, Minnesota, Wisconsin, Michigan, Ohio, and Pennsylvania, west of the Appalachian Mountains. Our evidence thus suggests that *viridis* monarchs keep to the central United States and *humistrata* monarchs keep to the eastern United States when migrating north. Perhaps the natural geographical barrier of the Appalachians causes this regional migration.

Even though we exerted substantially more field effort to collect monarchs in the southeast and northeast, we caught 6 times more monarchs in the more western samples from the south than in the southeast and 49 times more monarchs in the more western samples from the north than in the northeast. Hence, we suggest that our sample sizes reflect the regionality of monarch spring migration, from which we conclude that most monarchs are migrating from Mexico to Texas, where they breed extensively in early spring on the abundant *A. viridis* (see Cockrell et al., this volume; Lynch and Martin, this volume). Then their offspring migrate in very large numbers to the Great Lakes area. In contrast, considerably fewer monarchs remigrate from Mexico to Florida and correspondingly small numbers of their offspring appear to migrate north up the eastern coastal plain to the northeast. It is also possible that some of the spring butterflies in Florida may have overwintered in Florida along the

**Table 7.** Comparison of the wing conditions in southern spring (South) and northern spring (North) migrant monarchs. Samples are compared by a Wilcoxon two-sample test.

Monarch sample	N	Wing condition		Test statistic	
		Mean	SD	z	p
South	133	3.28	0.83	10.98	<0.0001
North	646	2.43	0.78		



**Figure 8.** Wing wear distributions of (a) western and eastern monarch samples from the south and (b) western and eastern monarch samples from the north of the United States (1 = fresh wings in pristine condition, in 0.5 intervals to 5 = extremely worn and tattered wings). The samples are compared statistically in Table 8.

coast of the Gulf of Mexico (Fig. 1), where small and mobile overwintering colonies occur (Brower, 1985).

As monarchs remigrate into Texas each spring they encounter the widely distributed and abundant milkweeds *A. viridis* and *A. asperula capricornu* (Fig. 1; Lynch and Martin, this volume). These two

**Table 8.** Regional comparisons of wing conditions in spring migrant monarchs from the western and eastern subsamples of monarchs from the south and north of the United States, east of the Rocky Mountains. Samples are compared by Wilcoxon two-sample tests with significance at 5%.

Monarch sample	N	Wing condition		Test statistics	
		Mean	SD	<i>z</i>	<i>p</i>
South					
Western	114	3.29	0.82	0.29	0.78 NS
Eastern	19	3.24	0.86		
North					
Western	633	2.44	0.79	2.70	0.007
Eastern	13	1.92	0.40		

plants are chemically and morphologically similar and are known to hybridize in their limited region of sympatry from central Texas to Oklahoma (Lynch and Martin, 1987, this volume; Martin and Lynch, 1988). Since no other milkweed species approaches the abundance of these two, it is curious that we have failed to identify *A. asperula*-derived monarchs in our field-collected samples. Two explanations may account for this discrepancy. Either the majority of our northern samples (from Minnesota, Wisconsin, and Michigan) were collected too far east to include *A. asperula* monarchs (which may migrate due north to the Dakotas), or we have failed to distinguish between the *A. viridis* and *A. asperula* fingerprints. Although the cardenolide fingerprints of these two species appear very distinct in the analyses of Lynch and Martin (1987) and Martin and Lynch (1988), the authors claim strong similarity in the two patterns. Furthermore, our *A. viridis* fingerprint from Florida (Fig. 3) is similar to that of *A. asperula* from Texas (Martin and Lynch, 1988, fig. 4). At present we cannot distinguish between these two alternative explanations and further work is needed for their resolution.

In addition to showing that the host-plant recolonization strategy of the monarch butterfly is predominantly one of successive brood migration, our data contradict prior generalizations about the physiology of insect migration and reproduction. Thus, previous authors (Johnson, 1960, 1969; Kennedy, 1961, 1985) have suggested that the reproductive physiology of migratory insects is repressed during migration, much like the reproductive physiology of nonmigratory insects that diapause and overwinter *in situ* (Rogers, 1983). Such reproductive repression in migratory insects is commonly referred to as the "oogenesis-flight syndrome" (Johnson, 1969; Dingle, 1980; Rankin et al., 1986) and reproduction occurs only when migratory behavior has ended with the arrival of a migrant at an appropriate resource site. The monarch butterfly clearly violates this syndrome since both overwintered butterflies and the subsequent first-generation butterflies lay eggs on available milkweeds as they migrate from Mexico to southern Canada. Furthermore, monarchs begin to mate before they leave the Mexican overwintering sites (Brower, 1985; Van Hook, this volume), and females almost certainly can lay eggs at any time or location along their northward migration. Although the oogenesis-flight syndrome does appear to exist in autumn migrant monarchs, on their way south to overwintering sites, Rankin (1985) and Rankin et al. (1986) found that it was much weaker in field-collected monarchs, especially in spring, than in laboratory-reared monarchs (see also Herman, 1985, this volume). This suggests that the oogenesis-flight syndrome is a dynamic phenomenon in monarchs that may or may not exist according to the interplay between the cues used to switch the syndrome on or off. The syndrome may be stronger in the autumn when monarchs migrate south to nonreproductive over-

wintering sites, and weaker, or absent in the spring when the same monarchs migrate north, especially since the spring remigrants need to lay eggs on the southern milkweeds they encounter. Thus, rather than definable in physiological terms (Kennedy, 1985), monarch migration is primarily an ecological phenomenon in which evolution has struck a dynamic balance between the benefits for monarchs in finding the best food resources for their larvae and the costs of carrying eggs and finding mates. The next generation faces the same problems, but the abiotic daylength and temperature cues in May (see Cockrell et al., this volume), to which an oogenesis-flight syndrome might respond, are weak and so we should expect a correspondingly weak or nonexistent syndrome. Nevertheless, the first generation of spring monarchs must be migratory in order to colonize the superabundant milkweed resource of *A. syriaca* in the north. These monarchs may well minimize the costs of carrying heavy, mature eggs and spermatophores by laying eggs as fast as they develop while migrating northward.

In conclusion, the differences between the cardenolide concentrations, fingerprints, and wing wear of southern and northern monarchs are definitive evidence for successive brood migration. These differences are also interesting in terms of the use of cardenolides by monarchs in their aposematic defense against avian predation (Brower, 1984). Southern, overwintered monarchs with low cardenolide concentrations will be poorly protected against naive bird predators, which could result in high mortality of these spring remigrants. However, these monarchs arrive before the major fledging period of immature and naive bird predators and so they are likely to encounter experienced, mature birds that may ignore them as aposematic prey. In contrast, the northern monarch arrivals, with high cardenolide contents, will be well protected against experienced and also naive birds, of which the first broods will be fledging at the time of arrival of these toxic monarchs in late May and early June (cf. Waldbauer and Sheldon, 1971; Waldbauer, 1988).

In comparison with other danaid species, a large investment in chemical defense is highly characteristic of the monarch (Ackery and Vane-Wright, 1984; Brower, 1984; Ackery, this volume; Schneider, this volume) and so predation is likely to be a strong selective force in shaping and maintaining the monarch's life history. In fact, the remarkable distinction between the cardenolide contents of southern and northern spring migrants may contribute strongly to the maintenance of selection for monarch migration (Malcolm and Brower, 1989). Since monarchs can tolerate the moderate freezing temperatures of their overwintering sites (Anderson and Brower, this volume) it is curious that they have not evolved sufficient freeze tolerance to diapause within the distributions of abundant milkweeds like *A. viridis* and *A. syriaca* and eliminate the costs of migration (Baker, 1978). However, monarchs are



also forced out of the southern states each summer by the onset of lethally high temperatures after completing, at most, two spring generations (Malcolm et al., 1987; Cockrell et al., this volume). By migrating farther north to *A. syriaca* (Fig. 1), monarchs can complete an additional three summer generations on this host plant (Malcolm et al., 1987) and maximize the number of autumn migrants that journey to Mexican overwintering sites. Thus, we suggest that an annual cycle of well-defended, first-generation spring migrants arriving in the northern United States, followed by less well-defended, breeding nonmigrants and then similarly defended autumn migrants, would allow monarchs to exploit any of their *Asclepias* host plants, in both space (from northern Mexico to southern Canada) and time (from late March to September). The weakest link in this cycle is the apparent loss of cardenolides during migration (Malcolm and Brower, 1989) resulting in the relatively poor defense of aggregated, overwintering monarchs in Mexico that suffer considerable predation from both birds and mice (Calvert et al., 1979; Fink and Brower, 1981; Fink et al., 1983; Brower and Calvert, 1985; Brower and Fink, 1985; Brower et al., 1985; Glendinning et al., 1988; Glendinning, this volume; Arellano G. et al., this volume).

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# Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence

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**ABSTRACT** Each year, millions of monarch butterflies from eastern North America migrate to overwinter in 10–13 discrete colonies located in the Oyamel forests of central Mexico. For decades efforts to track monarch migration have relied on observations and tag-recapture methods, culminating with the discovery of the wintering colonies in 1975. Monarch tag returns from Mexico, however, are few and primarily from two accessible colonies, and therefore tag-recapture techniques have not quantified natal origins or distinctiveness among monarch populations at wintering sites. Such information would be invaluable in the conservation of the monarch and its migration phenomenon since the wintering sites currently are threatened by habitat alteration. Here we show that stable hydrogen ( $\delta D$ ) and carbon ( $\delta^{13}C$ ) isotope ratios of wintering monarchs can be used to evaluate natal origins on the summer breeding range. Stable-hydrogen and carbon isotopic values of 597 wintering monarchs from 13 wintering roost sites were compared with isotopic patterns measured in individuals at natal sites across their breeding range over a single migration cycle. We determined that all monarch wintering colonies were composed of individuals originating mainly from the Midwest, United States, thereby providing evidence for a panmictic model of wintering colony composition. However, two colonies showed more northerly origins, suggesting possible priority colonies for conservation efforts.

More than 100 million monarch butterflies (*Danaus plexippus*) migrate annually from eastern North America to overwinter in 10–13 discrete (<2 ha) colonies located in remote Oyamel forests of central Mexico (refs. 1–6; Fig. 1). Monarchs returning to these wintering sites are separated by several generations from those that left the previous year, yet the same overwintering locations are used every year. Although monarch migration has been studied for more than 50 years (1, 6), how butterflies navigate to these locations from specific areas in North America (7) and whether colony composition is panmictic have long been mysteries.

Previously, monarch migration to Mexico has been studied by using tag-recapture techniques, which involves the placement of small identification tags on monarchs captured and released in the breeding range (ref. 3; Monarch Watch Organization, University of Kansas, <http://MonarchWatch.org>). Hundreds of thousands of monarchs have been tagged over the past five decades, with only 125 recoveries from Mexico occurring since 1975 (Monarch Watch Organization, University of Kansas, <http://MonarchWatch.org>). Most (83%) tags recovered between 1975 and 1998 were found at colonies with public viewing or research access (El Rosario and Sierra Chincua), with few or no tags recovered from the remaining

11–12 remote sites (Monarch Watch Organization, University of Kansas, <http://MonarchWatch.org>). Other difficulties include the fact that most tagged migrants are recaptured within the breeding range, and the number of individuals tagged does not reflect monarch production in any given area. Thus, tagging has not yielded quantitative information on proportions of monarchs originating from various parts of the breeding range. Clearly, a better tool is needed to assess the origins of wintering monarchs in Mexico. Such knowledge is required to focus conservation efforts in critical portions of the North American breeding range and at wintering sites in Mexico.

Based on laboratory and extensive field-rearing experiments across the eastern breeding range we showed that the stable-hydrogen ( $\delta D$ ) and carbon ( $\delta^{13}C$ ) isotopic composition of adult monarch wing membranes closely resembles, and permanently records, the isotopic composition of its natal (larval) food source (15). The isotopic composition of the monarch larval milkweed host plant (*Asclepias* sp.) is, in turn, controlled by continental isotopic patterns in rainfall for  $\delta D$  (8, 15) and other climatic and physiological factors for  $\delta^{13}C$  (8, 9). The  $\delta D$  and  $\delta^{13}C$  values of adult monarch wings from natal sites show isotopic trends across eastern North America, with increasingly depleted  $\delta D$  values found toward the northerly limits of the breeding range and  $^{13}C$  enrichment toward more northerly latitudes (15) (Fig. 2). These results show that natural signals of  $\delta D$  and  $\delta^{13}C$  are intrinsic markers of natal origins of monarchs. By sampling and measuring the isotopic composition of wintering monarchs in Mexico, we reasoned that it should be possible to infer their geographic natal origins and to establish whether wintering colonies are composed of monarchs from different regions.

## METHODS

We measured  $\delta D$  and  $\delta^{13}C$  values of 597 monarch butterflies collected from natural mortality at 13 wintering colonies (44–50 per colony) during February of 1997 (Table 1). These were individuals that migrated from North America in late summer and fall of 1996, and so could be related directly back to our 1996 breeding range isotopic study (15) (Fig. 2). Fifty male and 50 females were taken at random from each colony and stored in paper envelopes. Monarch wing membranes were separated from the abdomen, placed in glass vials, solvent-cleaned, air-dried, and stored.

Because a portion of the total hydrogen of monarch wing membrane (largely keratin) is available for isotopic exchange with ambient water vapor, it was necessary to quantify and eliminate the effect of this uncontrolled, temperature-dependent variable. Unfortunately, complete elimination of exchangeable hydrogen (i.e., hydrogen involved in O—H bonds) by chemical techniques such as nitration is not possible for complex organic matter (10). Hydrogen–isotope exchange

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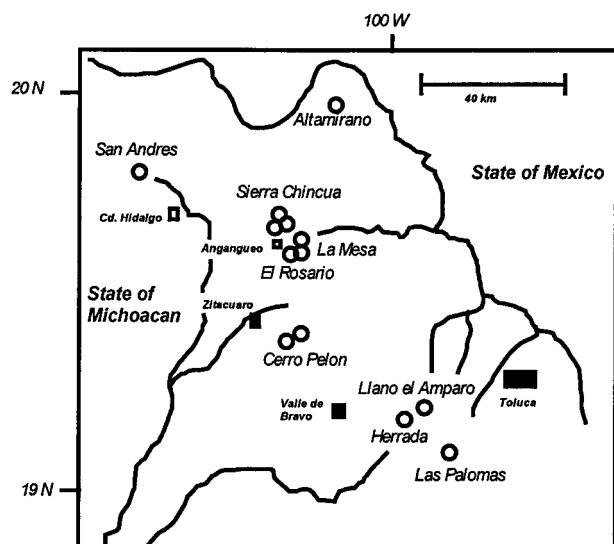


FIG. 1. Location of the 13 monarch wintering colonies (circles) in the Mexican States of Michoacán and México.

between wing membrane and water vapor was first quantified by equilibrating samples with steam having a wide range of hydrogen-isotopic values ( $-135$  to  $+525\text{‰}$ ) at constant temperature ( $130 \pm 0.1^\circ\text{C}$ ) and then measuring the total hydrogen  $\delta\text{D}$  values (10, 15). Hydrogen in monarch wing membranes

available for isotopic exchange at this temperature was determined to be  $19.5 \pm 0.7\text{‰}$  ( $r^2 = 0.99$ ,  $P < 0.001$ ,  $n = 27$ ). In all our samples, potential variability resulting from uncontrolled hydrogen-isotopic exchange was eliminated by controlled equilibration of all wing membrane samples with steam ( $\delta\text{D} = -135\text{‰}$ ) at  $130 \pm 0.1^\circ\text{C}$  for 2 hr. Sample reproducibility of repeated equilibrated samples was better than  $\pm 2\text{‰}$  for  $\delta\text{D}$ . Thus, total hydrogen-isotopic results for equilibrated samples could be compared reliably among samples and sites. After steam equilibration in Vycor break-seal tubes, all water vapor was cryogenically removed, and samples were sealed under vacuum, combusted at  $850^\circ\text{C}$  in the presence of cupric oxide, and followed by cryogenic separation of  $\text{CO}_2$  from  $\text{H}_2\text{O}$ . Waters of combustion were reduced to  $\text{H}_2$  gas on hot zinc (15). Stable-isotope analyses were performed on a Micromass Optima dual-inlet isotope-ratio mass spectrometer. Stable-carbon isotope analyses are reported in parts per thousand ( $\text{‰}$ ) deviation from the Pee Dee belemnite (PDB) standard, with a sample reproducibility of better than  $\pm 0.1\text{‰}$ . Stable-hydrogen isotope results are reported in parts per thousand deviation from the SMOW standard and normalized on the Vienna Standard Mean Ocean Water/Standard Light Antarctic Precipitation (VSMOW/SLAP) scale, with a sample reproducibility of better than  $\pm 2.0\text{‰}$ .

## RESULTS AND DISCUSSION

No effect of sex on the distribution of stable isotopes in monarch butterflies was observed (MANOVA  $F_{2,557} = 1.6$ ,

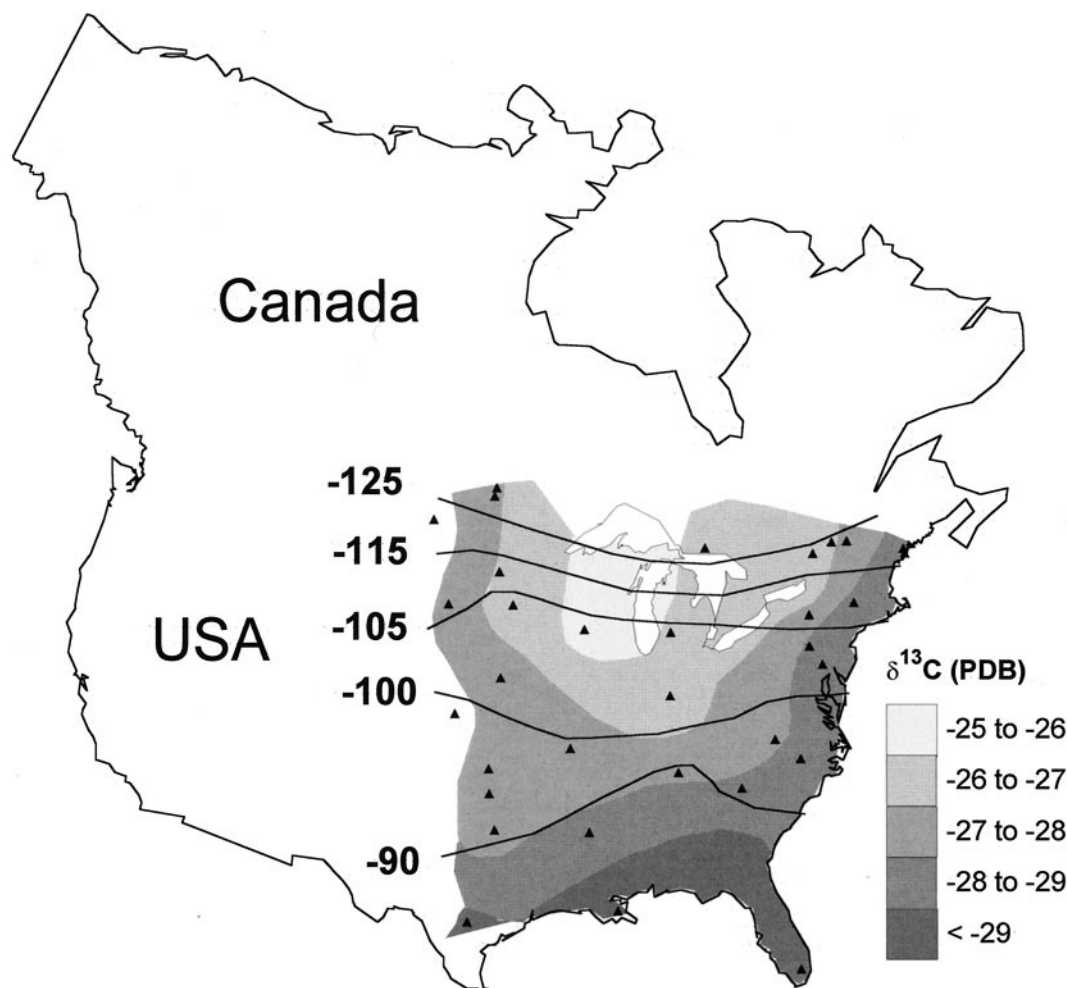


FIG. 2. Geographic patterns of  $\delta\text{D}$  and  $\delta^{13}\text{C}$  in monarch wings from natal sites across the breeding range of eastern North America (synthesized from ref. 15). Solid triangles depict field-rearing sites.

Table 1. Mean and 95% confidence interval (CI) for  $\delta D$  and  $\delta^{13}C$  values of monarch butterflies collected from 13 wintering colonies in Mexico

Wintering colony	$\delta D$ , ‰	95% CI, ‰	n	$\delta^{13}C$ , ‰	95% CI, ‰	n
Llano el Amparo	$-104 \pm 11$	-107 to -100	47	$-27.6 \pm 1.1$	-27.9 to -27.3	47
Altamirano	$-104 \pm 8$	-106 to -101	45	$-27.6 \pm 1.1$	-27.9 to -27.3	47
Sierra Chincua <i>Barranca Hondon</i>	$-110 \pm 10$	-113 to -107	44	$-27.5 \pm 1.3$	-27.9 to -27.2	47
Sierra Chincua <i>Llano el Toro</i>	$-105 \pm 8$	-108 to -103	46	$-27.3 \pm 1.1$	-27.7 to -27.0	48
Sierra Chincua <i>Barranca la Meurto</i>	$-106 \pm 11$	-110 to -103	48	$-27.4 \pm 0.9$	-27.7 to -27.2	50
Cerro Pelon <i>La Gota de Agua</i>	$-110 \pm 8$	-112 to -107	45	$-27.7 \pm 1.3$	-28.1 to -27.3	48
Cerro Pelon <i>Los Cedrales</i>	$-107 \pm 9$	-109 to -104	45	$-27.6 \pm 1.4$	-28.0 to -27.2	49
Herrada	$-106 \pm 10$	-109 to -103	48	$-27.5 \pm 1.1$	-27.8 to -27.2	48
Las Palomas	$-104 \pm 9$	-107 to -101	48	$-27.4 \pm 1.1$	-27.8 to -27.1	46
La Mesa	$-104 \pm 9$	-107 to -102	49	$-27.4 \pm 1.3$	-27.8 to -27.0	48
El Rosario <i>El Campanario</i>	$-108 \pm 11$	-111 to -104	48	$-27.7 \pm 1.0$	-28.0 to -27.4	50
El Rosario <i>Planos de los Conecos</i>	$-106 \pm 11$	-110 to -103	44	$-27.6 \pm 1.2$	-28.1 to -27.4	49
San Andres	$-106 \pm 10$	-109 to -103	45	$-27.7 \pm 1.2$	-28.1 to -27.4	50

Italicized sites refer to discrete subcolonies of monarchs that are generally considered part of the larger colony indicated.

$P = 0.2$ ), and populations from all colonies showed normal distributions for both  $\delta^{13}C$  and  $\delta D$  (Kolmogorov–Smirnov,  $P < 0.01$  in all cases). In general,  $\delta D$  and  $\delta^{13}C$  values of monarch wings overlapped considerably among wintering colonies (Ta-

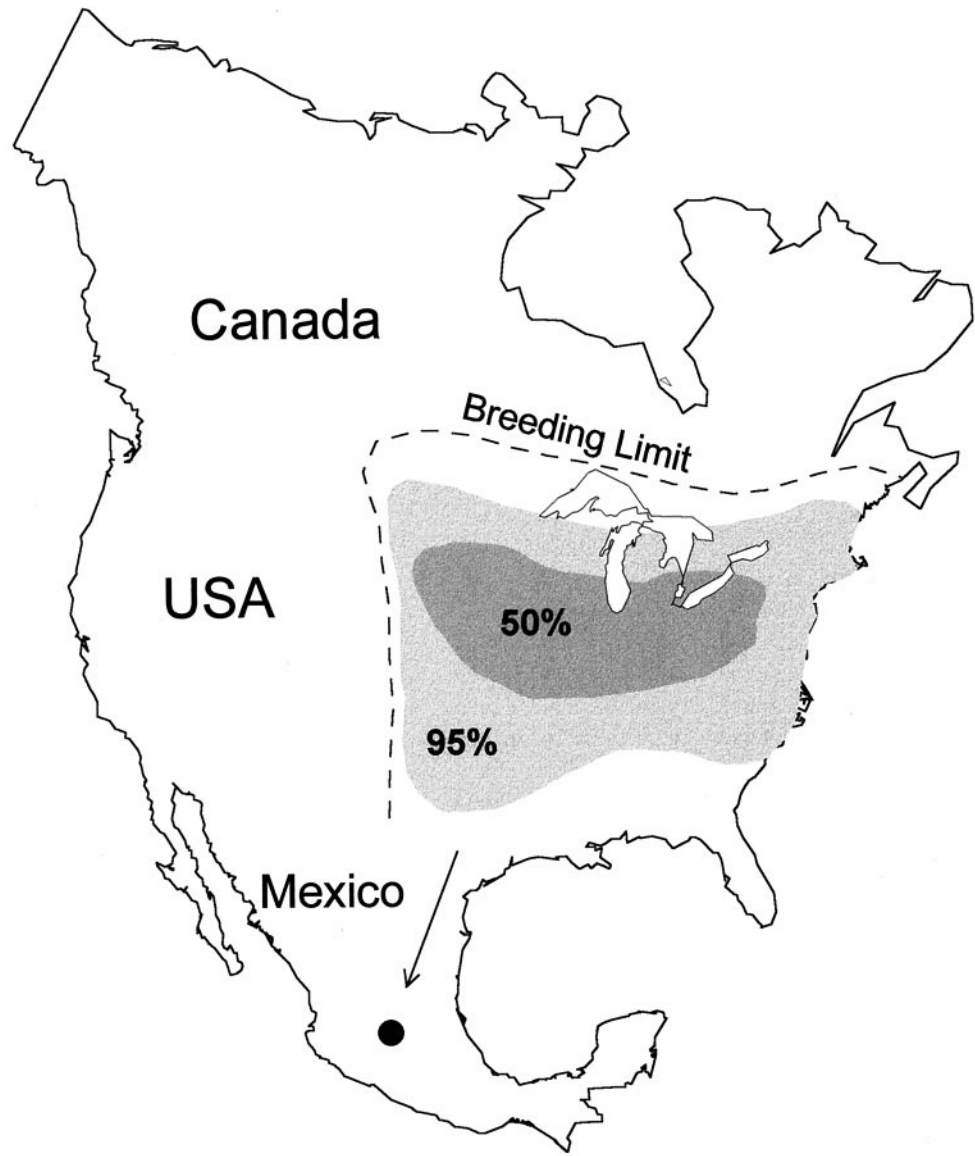


FIG. 3. Natal origins of monarch butterflies wintering in Mexico derived from  $\delta D$  and  $\delta^{13}C$  data ( $n = 597$ ). The light-gray area indicates the range of natal origins for 95% of monarchs from all wintering sites. The darker-gray area indicates range of natal origins for 50% of monarchs from all wintering colonies. The dashed line is the approximate monarch breeding range limit. The Mexican monarch overwintering colonies are denoted by the solid circle.

ble 1, MANOVA  $F_{24,1114} = 1.5, P = 0.05$ ), but some difference in the distribution of  $\delta D$  values among sites (ANOVA  $F_{12,558} = 2.2, P = 0.01$ ) was apparent (Table 1). The Barranca Hondon and La Gota de Agua monarch colonies were more depleted in  $\delta D$  values and did not overlap at the 95% confidence interval with the more enriched  $\delta D$  distributions of Llano el Amparo, Altamirano, Las Palomas, and La Mesa, suggesting more northern natal origins of monarchs at these two colonies.

We inferred natal origins of wintering monarchs from Mexico by comparing  $\delta D$  and  $\delta^{13}C$  data of wintering individuals with isotopic values found in monarchs field raised at natal sites throughout their breeding range (Fig. 2). Our  $\delta D$  and  $\delta^{13}C$  data show that 95% of wintering monarchs originated from throughout the known breeding range (Fig. 3). However, 50% of wintering monarchs originated from a fairly restricted geographic part of the breeding range, including the states of Kansas, Nebraska, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, and Ohio. This corresponds to an area of intense corn, soybean, and dairy production in the midwestern United States. Possibly, larger numbers of monarchs are produced in such areas of cultivation since milkweed host plants are persistent there despite pesticide and weed control measures. Proportionately fewer monarchs originated from the most southern or northern reaches of the breeding range (Fig. 3). Fewer wintering monarchs from the extremes of the breeding range were expected since relatively fewer individuals are produced there. Lack of representation from southern parts of the breeding range also is a result of the lack of larval host plant availability in the southern United States in late summer (11).

Our isotopic evidence shows that the 13 discrete monarch wintering colonies in Mexico generally are well mixed and thus demonstrates the existence of a panmictic model of monarch wintering colony composition. With the exception of the two sites noted, our results further suggest that the loss of a single wintering roost site is unlikely to affect one part of the breeding population in eastern North America over another. However, because the primary geographic production area for monarchs is centered in the American Midwest, that area should be focal for conservation efforts in North America. The combination of  $\delta D$  and  $\delta^{13}C$  measurements of tissues of breeding and winter-

ing populations of monarchs measured in a single year represents a new and powerful tool for understanding the ecology of this species and avoids interannual isotopic variability (12). Furthermore, our approach can be readily applied to other migratory organisms in North America and likely elsewhere and possibly refined through the assay of isotopes of other elements (13, 14).

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# Occurrence of common milkweed (*Asclepias syriaca*) in cropland and adjacent areas

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## Abstract

Interest in the population dynamics and geographic distribution of common milkweed (*Asclepias syriaca* L.) has recently increased due to the importance of common milkweed in the life cycle of the monarch butterfly (*Danaus plexippus*). A survey of common milkweed occurrence in various habitats was conducted in Iowa in June and July of 1999. Common milkweed was found in 71% of the roadsides and approximately 50% of the corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr.) fields. Corn and soybean fields had 85% fewer patches than roadsides. Conservation reserve program fields had the greatest average area infested. While common milkweed was frequently found in corn and soybean fields, average frequency and patch sizes were much greater in noncrop areas. © 2000 Elsevier Science Ltd. All rights reserved.

**Keywords:** Bt pollen; Monarch butterfly; Weed distribution; Maize; Soyabean

## 1. Introduction

Common milkweed is native to northeastern and north central United States and adjacent areas of Canada (Bhowmik and Bandeen, 1976). This perennial dicotyledonous species is present in crop and pasture lands of many areas, including Iowa. Common milkweed and other members of the Asclepiadaceae family are the sole food source of monarch butterfly larvae (Brower, 1969), whereas the adults feed on a wide range of flowers. Common milkweed is the most prevalent *Asclepias* species and is considered the preferred food source for monarch larvae in Iowa. The importance of common milkweed populations in the central United States to the monarch (Wassenaar and Hobson, 1998) and toxicity of the pollen of Bt (*Bacillus thuringiensis*) transformed-corn deposited on common milkweed leaves (Hansen and Obrycki, 1999; Losey et al., 1999) to monarch larvae has generated interest in the distribution of common milkweed in crop lands and adjacent areas.

Common milkweed is adapted to a wide range of climatic and edaphic conditions (Bhowmik and Bandeen, 1976). Infestations may be found under a wide range of soil conditions in any textural group, but are most prevalent on well-drained soils of loamy texture. In a roadside survey, Cramer and Burnside (1982) found that infestation frequencies in Nebraska varied greatly by crop and land use. Over 70% of the soybean, oat, (*Avena sativa* L.) and sorghum [*Sorghum bicolor* (L.) Moench.] fields were infested with common milkweed, while infestation frequencies for corn, wheat (*Triticum aestivum* L.), and alfalfa (*Medicago sativa* L.) were 36, 28, and 6% respectively. Railroad, roadside, and pasture areas had infestation percentages of 70, 51, and 14%.

Corn pollen may be dispersed 60 m or more by wind (Raynor et al., 1972) and deposited on other plants in and around corn fields and can be ingested by non-target organisms that feed on these plants. Recent research (Hansen and Obrycki, 1999; Losey et al., 1999) has shown that the expression and dispersal of the Bt toxin in the pollen of transgenic corn may be toxic to non-target Lepidoptera species. In a laboratory assay, larvae of the monarch butterfly reared on common milkweed leaves dusted with pollen from Bt corn, ate less, grew more slowly, and had higher mortality than larvae reared on leaves dusted with untransformed pollen or on leaves

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without pollen (Losey et al., 1999). Hansen and Obrycki (1999) found that common milkweed leaf samples taken from within and at the edge of corn fields caused 19% mortality of monarch larvae with Bt pollen compared with 0% with non-Bt pollen and 3% in no pollen controls.

Each fall (autumn), millions of monarch butterflies from eastern North America migrate to 10–13 discrete colonies in the Oyamel forests of central Mexico (Wassenaar and Hobson, 1998). Although monarch migration has been extensively studied, the migration patterns to these locations from specific areas in North America has not been characterized. Using an isotopic labeling technique, Wassenaar and Hobson (1998) determined that all monarch wintering colonies were composed of individuals originating mainly from the Midwest United States. The importance of the Midwest in the monarch butterfly life cycle raises concerns over common milkweed populations in corn and soybean fields as the use of glyphosate-resistant crops increase. Glyphosate provides an additional herbicide for the control of common milkweed in the growing crop (Owen and Hartzler, 1999) and may reduce common milkweed occurrence in crop fields.

In areas of the North America where the landscape is dominated by row-crop production, a large proportion of the common milkweed may be either in corn/soybean fields or in adjacent areas within several meters of the field edge. The objective of this research was to determine the relative distribution of common milkweed in different habitats in Iowa. The results will help assess the potential impact of Bt pollen on monarch populations and proportion of milkweed that occurs in crop fields that may be affected by changing herbicide use patterns.

## 2. Materials and methods

Distribution and patch characteristics of common milkweed in Iowa were determined by a plant census conducted during June and July of 1999. To generate a random, representative sample of the state, a 10 km grid was superimposed on a map of the state of Iowa. The grid was generated using a Universal Transverse Mercator coordinate reference system for use with other digital map data in Arc/Info.<sup>1</sup> The grid was bisected horizontally and vertically to create four quarter grids. Within each quarter grid, ten 10 km grid cells were selected at random. The random selection was performed for each quarter using the Arc/Info RESELECT command with the RANDOM option. The selected grid

cells were mapped with the 1 : 100,000-scale US Geologic Survey Digital Raster Graphic map series for navigation and field map selection. If a 10 km grid cell was split between two 1 : 100,000-scale map sheets, the sample was shifted one cell south and two cells east.

Within each 10 km grid cell ten 0.5 ha (50 m × 100 m) sampling areas were surveyed for the presence of common milkweed. County plat maps were used to locate the sampling areas within grid cells. Sampling areas were identified prior to going to the field by arbitrarily specifying a distance from a landmark, usually a road intersection or railroad crossing. This method eliminated sampling bias since the sampling areas were selected without prior knowledge of terrain or vegetation. Each sampling area was immediately adjacent to the road, extending 100 m into the field. The majority of sampling areas consisted of publicly owned roadside adjacent to the road and a second area of private property. The width of roadside in most situations was approximately 10 m and the dominant vegetation was smooth brome (*Bromus inermis* Leyss.).

Each sampling area was divided into sub-samples (sites) based upon the land use (i.e., roadside and corn). Data collected included size of site, vegetation type, crop growth stage, number of distinct common milkweed patches, and size of individual common milkweed patches. Common milkweed stems within 1 m of each other were considered be part of a single patch, and patch size was estimated as the area encompassed by the contiguous stems. Solitary stems were assigned a patch size of 1 m<sup>2</sup>.

Data were analyzed using analysis of variance to allow comparison of common milkweed distribution among the different land uses. Data presented include the percentage of sites infested with common milkweed, mean number of patches per site, and cumulative area infested with common milkweed. Sites not infested with common milkweed were not included in the data set when calculating the mean number of patches and cumulative infestation. Paired *t*-tests were used to compare common milkweed patch frequency and area of infestation among land uses.

## 3. Results and discussion

A total of 859 sites were surveyed with roadside being the most common habitat sampled (Table 1). Corn and soybean were the next most common land uses followed by lower numbers of pastures, waterways and terrace areas, and conservation reserve program fields. Other land uses, including crops other than corn and soybean, railroad right-of-ways, wooded areas, grassed field corners, and fallow, comprised 7% of the observations and were treated as a single group in analysis of the data. Based on the design of the experiment and land use

<sup>1</sup> Source of materials: ESRI, Redlands, CA 92373. Reference to a trade or company name is for specific information only and does not imply approval or recommendation of the company by the USDA to the exclusion of others that may be suitable.



Table 1

Common milkweed occurrence, patch number, and estimated area infested by land use category in Iowa in 1999

Land use	Number of observations	Sites infested (%)	Number of patches <sup>1</sup> (no. 0.1 ha <sup>-1</sup> )	Area infested <sup>1</sup> (m <sup>2</sup> ha <sup>-1</sup> )
Roadside	407	71	4.8 a <sup>2</sup>	102 b
Corn	179	46	0.7 c	30 c
Soybean	153	57	0.7 c	16 d
Pasture	36	28	0.7 c	14 d
Waterways and terraces	13	46	1.7 b	169 ab
Conservation reserve program	15	67	0.9 c	212 a
Other <sup>3</sup>	56	41	2.0 b	61 bc

<sup>1</sup>Based on infested fields.<sup>2</sup>Means followed by the same letter within a column do not differ using paired *t*-tests at  $P \leq 0.05$ .<sup>3</sup>Other land uses included crops other than corn and soybean, railroad, wood lots, and grassed field corners.

patterns in Iowa, this was considered a representative sample of the landscape.

Common milkweed was found in 71% of the roadside sites, 46 and 57% of corn and soybean fields, 28% of pastures, 46% of the waterway and terraces sites, and 67% of conservation reserve program fields. Generally, noncropped lands had higher percent infestation than corn and soybean fields. Higher infestations of perennial plants are expected in undisturbed areas (Buhler, 1995). These infestation percentages are somewhat different from those observed in Nebraska (Cramer and Burnside, 1982) where 73% of the soybean fields and only 51% of the roadsides were infested.

Roadside areas had the highest frequency of common milkweed patches (4.8 0.1 ha<sup>-1</sup>) within infested sites (Table 1). Waterways and terraces and other land uses had the next highest frequencies of common milkweed patches, but the frequency of patches was 42% or less of that in roadsides. Frequency of patches in pastures and corn and soybean fields was only 15% of that in roadside areas. The frequent soil disturbance and herbicide application in corn and soybean fields would be expected to prevent rapid spread of common milkweed in areas producing annual crops (Bhowmik and Bandeen, 1976).

Conservation reserve program fields, waterways and terraced areas that were infested with common milkweed had the greatest average area infested, even though the number of patches was relatively low (Table 1). Average area per ha infested in roadsides was about 50% of conservation reserve program fields, but was still 3.4–6.4 times greater than the average infested area in corn and soybean fields. Undisturbed areas provide individual common milkweed plants greater opportunity to develop an extensive root system and develop larger patches (Bhowmik and Bandeen, 1976).

Common milkweed was ubiquitous in the state of Iowa. Infestation levels were lowest in agricultural land (corn, soybean and pasture), however, this land use encompasses 78% of the total Iowa land area (Tiffany and Miller, 1999). The 9 million ha planted to corn and

soybean annually may be an important refuge for monarchs. Therefore, changes in herbicide use patterns that result in more effective common milkweed control may impact monarch populations.

Roadsides had the highest infestation frequency of the vegetation types surveyed. There are approximately 330,000 ha of roadsides in Iowa. Although the total land area maintained in roadside vegetation is relatively small, the uniform distribution of roadsides across the landscape may increase their importance for the monarch butterfly. The narrow width of roadsides (10 m or less in most situations) places much of the common milkweed found in this habitat close to land planted to corn and soybeans. Monarchs that use these plants may be at risk if the adjacent corn fields are planted to Bt hybrids (Hansen and Obrycki, 1999; Losey et al., 1999).

This research provides information about the distribution of common milkweed in Iowa and areas with similar climate and land use patterns. However, additional information is required before an accurate assessment can be made of the potential impact of genetically modified crops on monarch butterflies.

## Acknowledgements

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# Temporal and spatial overlap between monarch larvae and corn pollen

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To assess the likelihood that monarch larvae will be exposed to *Bacillus thuringiensis* (Bt) pollen, we studied milkweed and monarch densities in habitats which comprise much of the land available to breeding monarchs, e.g., cornfields, cornfield edges, other agricultural fields, and nonagricultural areas, in four regions of the monarch breeding range. We found that monarchs use milkweed in cornfields throughout their breeding season, and that per plant densities are as high or higher in agricultural habitats as in nonagricultural habitats. As a result of the prevalence of agricultural land, most of the monarchs produced in the upper Midwest are likely to originate in cornfields or other agricultural habitats. There was a greater temporal overlap between susceptible monarchs and corn anthesis in the northern than the southern part of the summer breeding range, because of earlier pollen shed in the south. The importance of agricultural habitats to monarch production suggests that, regardless of the impact of genetically modified crops, agricultural practices such as weed control and foliar insecticide use could have large impacts on monarch populations.

Transgenic *Bacillus thuringiensis* (Bt) corn (*Zea mays* L.) plants were designed to control the European corn borer [*Ostrinia nubilalis* (Hübner)] and other Lepidoptera feeding on corn tissue. The Bt toxins produced by transgenic corn are lepidopteran-specific and kill only insects that ingest the plant tissue. Because of this specificity, the impact of Bt corn on nontarget organisms was assumed to be negligible (1–3). However, most commercial Bt hybrids express the endotoxin in their pollen to varying degrees and thus may impose risks to nontarget Lepidoptera that consume pollen deposited on their host plants (4, 5).

Many aspects of monarch butterfly (*Danaus plexippus* L.) biology may make them particularly susceptible to impacts of corn pollen exposure. Monarch larvae are present in the late summer, when a portion of the corn acreage is pollinating. A recent stable isotope study (6) suggested the Corn Belt is the origin of most monarchs that migrate to Mexico and form the nucleus of the following year's population. Malcolm *et al.* (7) estimated that 92% of the monarchs overwintering in Mexico had fed as larvae on *Asclepias syriaca* (common milkweed), a common weed in agricultural habitats (8–10).

To determine the potential impact of Bt corn on monarch populations, we need to know the proportion of the monarch population that overlaps temporally and spatially with corn pollen during the larva stage. To estimate this quantity, we measured relative monarch densities in different habitats and the temporal overlap between monarch larvae and corn anthesis. In addition, we compared survivorship of larvae in different habitats to determine whether oviposition patterns reflect monarch production from each habitat.

## Materials and Methods

**Study Sites.** The study was conducted in four regions representing different parts of the breeding range of eastern North American monarchs: (i) east central Minnesota and west central Wisconsin, (ii) central Iowa, (iii) coastal Maryland, and (iv) southern Ontario.

Research groups in each region monitored five sites where different habitat types were represented (Table 1). All sites included a field planted in nontransgenic corn (except Maryland and Iowa, where one site contained transgenic corn) and most included a nonagricultural area. Nonagricultural areas were neither planted in crops nor highly urban, and included old fields, restored prairies, or pastures. In addition, Minnesota/Wisconsin, Iowa, and Maryland sites included 10-m belts at the edge of cornfields (encompassing roadsides and fencerows), and Iowa and Maryland sites included agricultural fields other than corn. All habitats at a given site were within 1 km of each other to increase the chance that the same monarch population was exposed to all habitats within a site. Only cornfields containing at least 10 milkweed ramets (aboveground stems) per hectare were included to ensure sufficient opportunity to observe monarchs.

**Weekly Monitoring of Monarch Abundance.** The monitoring procedure was adapted from the Monarch Larval Monitoring Project, a volunteer program initiated in 1997 (ref. 11; www.monarchlab.umn.edu). We monitored U.S. sites weekly from late May or early June through August, when monarchs were present in each location, and the Ontario site in July and August. In each habitat, we searched a large number of milkweed ramets and recorded monarch egg and larval presence on a per ramet basis. In most habitats, we examined ramets along randomly selected belt transects or monitored all ramets in smaller plots within the habitat. In habitats where milkweed density was too low to encounter 200 ramets on transects, we sampled all ramets. The number of ramets sampled per week on transects ranged from 200–1,400, and in complete samples from 25–600. Instars were differentiated by using head-capsule size and tentacle length (12). The frequency and specificity (identifying larvae to instar) of our protocol meant that few individuals could be counted twice in the same stage; at normal summer temperatures, larvae hatch 4–6 days after oviposition, and individual stadia last from 2 to 5 days (13).

All research teams noted the weeks in which over 20% of corn plants were shedding pollen at each site and recorded the height of a random sample of 30 milkweed ramets in each habitat each week. In Iowa, Minnesota/Wisconsin, and Ontario, we measured heights of 10–30 corn plants at each site weekly.

**Estimating Milkweed Density.** Minnesota/Wisconsin and Ontario research teams quantified milkweed density (in ramets per m<sup>2</sup>) at each study site by sampling a minimum of 50 m<sup>2</sup> quadrats along randomly selected transects across the fields in corn and nonagricultural areas (transect lengths varied with the size of the

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**Table 1. Site characteristics**

Region	Site name	Cornfield size, acres	Anthesis dates (>20% of corn-shedding pollen)	Habitats surveyed	Coordinates
Minnesota/ Wisconsin	Farm	40	July 31–August 7	Corn, edge, nonagricultural	45.1N, 92.5W
	Amery	11	July 27–August 3	Corn, edge, nonagricultural	45.3N, 92.4W
	AG	170	July 25–August 1	Corn, edge, nonagricultural	45.1N, 92.5W
	Rosemount	23	July 21–28	Corn, edge, nonagricultural	44.7N, 93.1W
Iowa	Cedar Creek	1	July 19–26	Corn*, edge, nonagricultural	45.3N, 93.2W
	595/280	10	July 7–13	Corn, edge, soybean	41.9N, 93.5W
	Shipley S	180	July 7–13	Corn <sup>†</sup> , edge, soybean	42.0N, 93.5W
	LincolnWay	80	July 11–17	Corn, edge, nonagricultural	42.0N, 93.7W
	Coopers	40	July 5–19	Corn, edge, soybean	42.0N, 93.7W
	Ankeny	30	July 6–12	Corn, edge, soybean, nonagricultural	41.7N, 93.6W
	Fergus	38	August 7–14	Corn, nonagricultural	43.4N, 80.2W
Ontario	New Hamburg	50	August 1–15	Corn, nonagricultural	43.2N, 80.4W
	Drayton	45	August 10–17	Corn, nonagricultural	43.5N, 80.2W
	Linwood	35	August 9–16	Corn, nonagricultural	43.5N, 80.3W
	Palmerston	100	August 4–18	Corn, nonagricultural	43.5N, 80.4W
	Wye Farm	45	July 19–31	Corn, edge, soybean, nonagricultural	38.5N, 76.1W
Maryland	Wye Island	33	July 6–17	Corn, edge, soybean, nonagricultural	38.5N, 76.1W
	Gannon	22	July 7–19	Corn, edge, soybean, nonagricultural	39.1N, 76.5W
	Airport	20	July 27–Aug 9	Corn <sup>†</sup> , edge, orchard, nonagricultural	39.1N, 76.5W
	Beaverdam	12	July 6–18	Corn, edge, forage crop, nonagricultural	39.0N, 76.6W

\*Sweet corn.

<sup>†</sup>Bt corn (event 176 in Maryland and Bt 11 in Iowa).

field), and a minimum of 30 quadrats in the smaller edge areas (14). In Maryland, we counted the total number of milkweeds at each study site and measured the area of the site by using aerial maps to determine milkweed densities.

Because our study sites were not necessarily representative of available habitats, we obtained landscape milkweed densities when possible. The Ontario team surveyed agricultural and nonagricultural sites in Huron, Kent, and Wellington counties, with 8 replicates of corn and 10 of nonagricultural areas in each county. Four transects (2 × 100-m) within each field were surveyed to determine the number of milkweed ramets per m<sup>2</sup>. The dimensions of each of site were at least 100 × 100 m. Fields were chosen by calling growers before the survey, avoiding bias for fields with high or low milkweed densities. The first nonagricultural habitats observed near the agricultural fields were surveyed, as long as they met dimension and composition criteria (freely growing open field). We used estimates of milkweed densities in Iowa from Hartzler and Buhler (10) for Midwestern study sites. Milkweed density in each habitat type was converted to the proportion of milkweed on the landscape by using data compiled by Taylor and Shields (15) for U.S. sites, and 1983 and 1984 land cover information (the latest dates for which data were available) from the Ontario Ministry of Agriculture, Food, and Rural Affairs (16, 17) for Canadian sites.

**Estimating Monarch Production from Each Habitat.** To estimate the proportion of monarch production that comes from cornfields, we multiplied three quantities: (i) relative monarch production per milkweed ramet in each habitat, (ii) relative milkweed ramet density in each habitat, and (iii) the proportion of the landscape composed of each habitat type. This product estimates the relative number of monarchs produced in each habitat type, and thus the proportion of monarchs produced in cornfields.

**Phenological Overlap.** We calculated the percentage of larvae present during corn anthesis by summing per plant densities of first and second instars observed in each cornfield during anthesis and dividing them by the sums of the per plant densities observed during the entire monitoring period. This calculation was done separately for each field, because anthesis dates within a region varied. We used densities rather than counts because the number of milkweed ramets searched varied during the moni-

toring period, and using counts would overrepresent dates on which more plants were searched. We included only first and second instars to avoid counting individuals more than once, as they are likely to still be larvae the following week.

**Statistical Analyses.** We used repeated-measure ANOVAs to compare monarch use of different habitat types. Separate ANOVAs were carried out for each region, with the different sites within a region acting as replicates. The response variable was the number of eggs per milkweed ramet (arcsine transformed to normalize data), with repeated measures by date. Habitat type, site, and date were main effects. The error term for habitat and site main effects was the habitat × site interaction, and the error term for the date and date × habitat effects was the habitat × site × date interaction.

We calculated survival probability from the proportions of each instar observed relative to egg numbers in each habitat, using the Weibull frequency distribution to analyze survivorship data (18, 19). This analysis allows statistically and ecologically meaningful inferences from model parameters. The form of the model is  $S_P(t) = \exp[-(t/b)^c]$ ,  $t, b, c > 0$ , where  $S_P(t)$  is the probability of an individual surviving to age  $t$ ,  $b$  is a scaling parameter that indicates mortality (high  $b$  corresponds to low mortality), and  $c$  is a shape parameter ( $c = 1$  corresponds to constant mortality and  $c < 1$  corresponds to decreased mortality with age). Cumulative degree days needed to reach the end of each stage (13) were used for age intervals. We compared parameters within each region by using Welch's unpaired  $t$  test with a Bonferroni adjustment for multiple comparisons. Because pupae are rarely observed, and adults can leave their natal area within a few hours of eclosion, we could measure only survival during the larva period. Thus, our method assumes that survival after the larval stage does not vary among habitats.

## Results

**Egg Densities on Milkweeds in Different Habitats.** Monarchs oviposited on milkweed in all habitats throughout the summer (Fig. 1) and were consistently present in cornfields, even in late summer when milkweed ramets were much shorter than the surrounding corn (Fig. 2). Statistical analyses of the effects of habitat type and other variables on monarch densities are summarized in Table 2 (Table 4 summarizes per ramet egg densities over the entire summer).



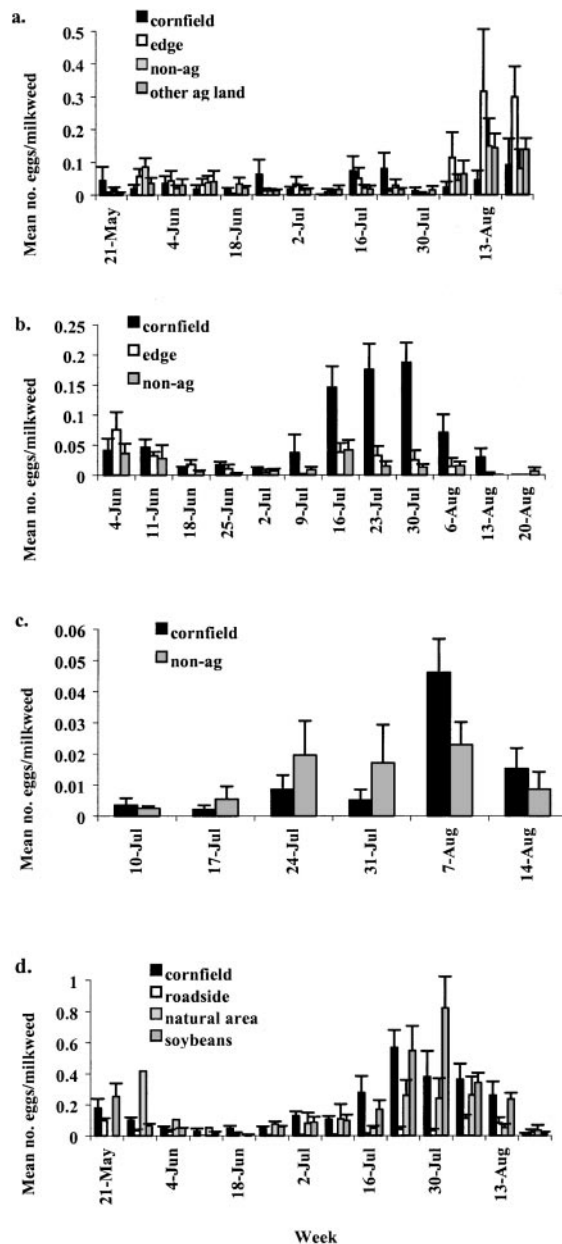


Fig. 1. Average egg densities over time for (a) Maryland, (b) Minnesota/Wisconsin, (c) Ontario, and (d) Iowa. Bars represent standard error. Date is the first day of the week in which data were collected. Note that y axis scale varies between regions.

Relative egg densities among different habitat types varied by location. In Maryland, where we compared corn, corn edge, and other agricultural and nonagricultural sites, there were no significant differences among habitat types. In Minnesota/Wisconsin, where we compared corn, corn edge, and nonagricultural habitats, there were significantly higher egg densities in cornfields than the other two habitats. In Ontario, where we compared cornfields and nearby nonagricultural sites, there were no significant differences in egg densities between the two habitat types. In Iowa, milkweed plants in agricultural areas (both corn and soybean fields) had higher egg densities than those in cornfield edges. Only two Iowa sites included a nonagricultural habitat, and a separate analysis of these two sites showed no habitat effects ( $P > 0.31$ ; data not shown). Significant date  $\times$  habitat interactions occurred in Minnesota/Wisconsin and Iowa, where relative egg densities in agricultural

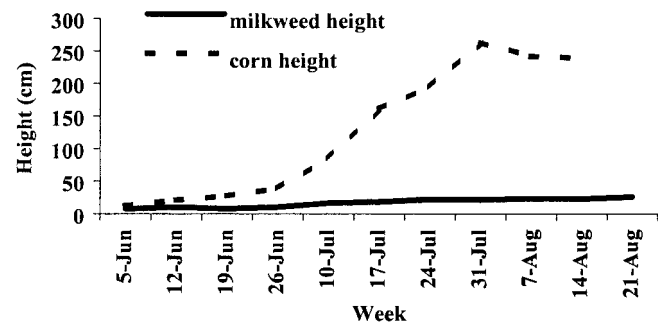


Fig. 2. Average height of milkweed and corn plants for one Minnesota/Wisconsin site. The pattern was similar in all regions, with corn at least twice the height of milkweed by early July.

habitats were higher late in the summer. The significant main effect of date at all sites results from separate peaks in monarch abundance (Fig. 1). There were no significant main effects of site in any region except Maryland, where only two sites differed from each other.

There was no correlation between per ramet egg densities and milkweed densities in surveyed Ontario and Minnesota/Wisconsin fields (data not shown).

**Larval Survival in Different Habitats.** Fig. 3 illustrates the relative numbers of each instar found in each habitat type over the entire summer, calculated as a proportion of the number of eggs observed. There were significant differences in the  $b$  parameter in the Weibull model (mortality rate) among habitats in all regions except Maryland (Table 3). In Minnesota/Wisconsin and Ontario, survival was higher in cornfields than other habitats; and in Iowa, survival was higher in corn and nonagricultural habitats than in edge habitats. There were no differences among habitats in the  $c$  parameter; in all regions and habitats there was higher mortality in the earlier stages ( $c < 1$ ). Despite mortality differences early in development, by the later stadia survival was similar across habitats (Fig. 3). The slight increase at the end of the survival curves in some regions may be the result of smaller sample sizes of the later stadia or higher apparency of fifth instar larvae.

#### Relative Contribution of Different Habitats to Monarch Production.

Calculations of the relative contributions of different habitats are summarized in Table 4. For each habitat within a region, we used the number of eggs observed throughout the season divided by the total number of plants observed to represent relative per ramet contributions to monarch production. We used egg densities rather than later-life stages because the higher sample sizes make these estimates of relative abundance most accurate, and because there were no habitat effects on survival to the later stadia. Milkweed density was generally higher in the nonagricultural habitats than cornfields in all regions (Table 4). This was true in both the monitored fields (Table 4, column 4) and other fields (Table 4, column 5). Because we had no estimates of milkweed density on a landscape basis for Maryland, as we did for Ontario and the upper Midwest, we did not estimate relative monarch productivity in Maryland. We had to survey over 30 fields in Maryland to find 5 that contained high enough milkweed densities to monitor, and are confident that densities in surveyed fields are higher than those in most Maryland cornfields.

Productivity on a per area basis, relative to nonagricultural areas, is shown in column 6, and relative contribution to the total population (taking both overall area and density into account) is shown in column 8 of Table 4. Estimates of productivity suggest that nonagricultural habitats in Ontario are  $\approx 2$  orders of magnitude more productive than cornfields on a per area basis. Nonagricul-



**Table 2. ANOVA of factors affecting egg densities**

Region	Source	df	Sum of squares	F	P
Maryland	Habitat	3	$5.54 \times 10^{-2}$	1.96	0.174
	Site (replicate)	4	$3.25 \times 10^{-1}$	8.61	0.002*
	Habitat $\times$ site	12	$1.13 \times 10^{-1}$		
	Date	6	$4.01 \times 10^{-1}$	10.62	0.000
	Date $\times$ habitat	18	$2.14 \times 10^{-1}$	1.86	0.029†
	Habitat $\times$ site $\times$ date	95	$5.98 \times 10^{-1}$		
Minnesota/Wisconsin	Habitat	2	$1.42 \times 10^{-1}$	28.68	0.002‡
	Site (replicate)	4	$1.89 \times 10^{-2}$	1.91	0.202
	Habitat $\times$ site	8	$1.98 \times 10^{-2}$		
	Date	6	$9.81 \times 10^{-2}$	9.96	0.000
	Date $\times$ habitat	12	$9.03 \times 10^{-2}$	4.58	0.000†
	Habitat $\times$ site $\times$ date	70	$1.15 \times 10^{-1}$		
Ontario	Habitat	1	$8.31 \times 10^{-6}$	0.02	0.892
	Site (replicate)	4	$1.31 \times 10^{-3}$	0.83	0.572
	Habitat $\times$ site	4	$1.58 \times 10^{-3}$		
	Date	5	$6.54 \times 10^{-3}$	6.05	0.000
	Date $\times$ habitat	5	$2.14 \times 10^{-3}$	1.98	0.103
	Habitat $\times$ site $\times$ date	40	$8.65 \times 10^{-3}$		
Iowa	Habitat	2	1.32	14.68	0.005‡
	Site (replicate)	3	$1.47 \times 10^{-1}$	1.06	0.433
	Habitat $\times$ site	6	$2.79 \times 10^{-1}$		
	Date	6	1.39	10.96	0.000
	Date by habitat	12	$7.34 \times 10^{-1}$	2.58	0.005†
	Habitat $\times$ site $\times$ date	47	1.01		

"Habitat" main effect refers to different habitat types, and "site" refers to locations within a region. "Date" is included as a categorical variable. Significant main and interaction effects were compared by using Bonferroni tests.

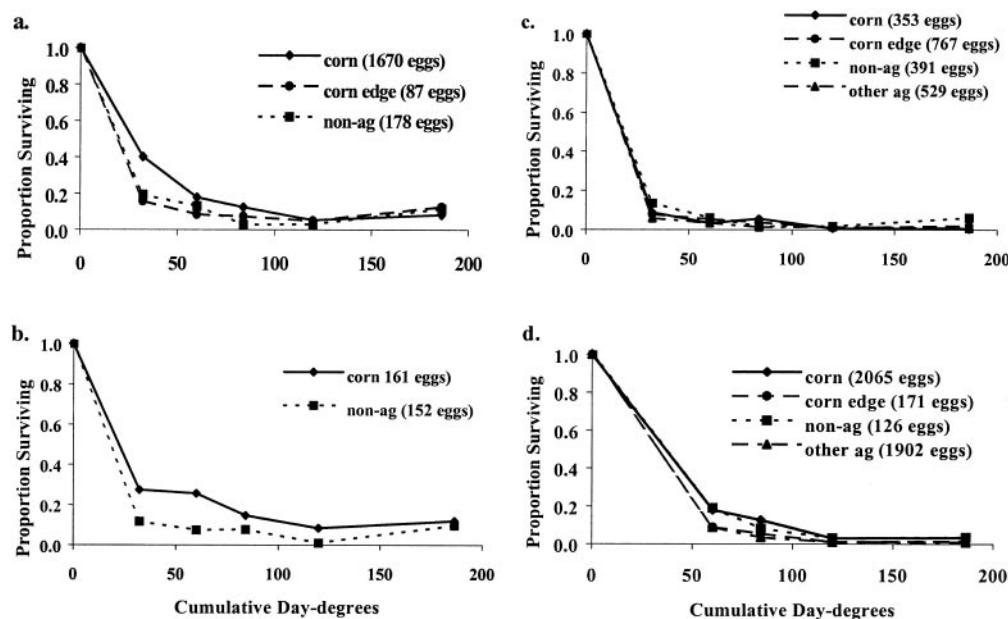
\*Maryland airport site had higher egg densities than Wye Island site.

†Significant date  $\times$  habitat interactions in Maryland are caused by higher egg densities in edge sites 21–25 August, in Minnesota/Wisconsin by higher densities in cornfields 17 July–5 August, and in Iowa by higher densities in corn and bean fields 24 July–4 August.

‡Minnesota/Wisconsin cornfields had higher egg densities than edge or nonagricultural habitats. Iowa corn and bean fields had higher densities than edge habitats.

tural habitats were about 2 and 4 times as productive as cornfields in Minnesota/Wisconsin and Iowa, respectively. When the amount of landscape that includes agricultural and nonagricultural land is taken into consideration, we estimate  $\approx 73$  (in Minnesota/Wisconsin) or 45 (in Iowa) times more monarchs come from cornfields than from nonagricultural habitats. In Ontario, we estimate that cornfields produce  $\approx 18$  times fewer monarchs than nonagricultural areas. The overall contribution of field edges cannot be estimated because of a lack of data on their relative areas.

**Phenological Overlap Between Monarch Larvae and Pollen Shed.** In Minnesota and Ontario, there was substantial overlap (40% and 62%, respectively) between the peak of the migratory monarch generation and pollen shed, whereas pollen shed occurred before the peak of the final generation in Iowa and Maryland (15% and 20% overlap, respectively; Fig. 1 and Table 1 show egg peaks and anthesis dates). Estimates of the proportion of first and second instar larvae in cornfields that are exposed to corn pollen are shown in Table 5.



**Fig. 3.** Survival calculated as the proportion of each stage observed relative to the number of eggs observed in (a) Minnesota/Wisconsin, (b) Ontario, (c) Maryland, and (d) Iowa. Egg numbers are in parentheses. Data on first instars were not used in Iowa calculations because of inconsistencies in identifying this life stage.

**Table 3. Weibull parameters comparing survival in different habitats**

Region	Habitat type	<i>b</i>	SE	<i>c</i>	SE
Minnesota/ Wisconsin	Corn	34.20*	3.725	0.8111	0.1330
	Edge	7.15 <sup>†</sup>	4.331	0.3771	0.1020
	Nonagricultural	10.08 <sup>†</sup>	4.011	0.4340	0.0951
Iowa	Corn	30.55*	9.390	0.7850	0.2550
	Edge	16.68 <sup>†</sup>	3.988	0.6880	0.1120
	Nonagricultural	36.07*	8.018	1.0290	0.3235
	Other agricultural	22.74* <sup>†</sup>	3.198	0.9187	0.1186
Maryland	Corn	5.78*	2.084	0.4970	0.0861
	Edge	5.29*	1.480	0.4803	0.0613
	Nonagricultural	8.32*	4.458	0.5198	0.1580
	Other agricultural	5.04*	1.750	0.5383	0.0890
Ontario	Corn	19.68*	6.304	0.4045	0.0944
	Nonagricultural	6.00 <sup>†</sup>	3.674	0.4022	0.1059

Welch's unpaired *t* test with Bonferroni adjustment used for multiple comparisons of model parameters within each region. Values followed by the same superscript are not significantly different at the adjusted confidence level ( $P < 0.05$  or less, depending on number of comparisons).

## Discussion

**Importance of Agricultural Fields to Monarch Production.** We found immature monarchs in cornfields throughout their breeding season, even though the corn is up to 200 cm taller than the milkweed by the end of the season (Fig. 2). In the four regions studied, per ramet densities of monarchs were as high or higher within cornfields as in other habitats on many monitoring dates. Egg densities on milkweed in agricultural fields were even higher relative to nonagricultural habitats at the end of the summer in the upper Midwestern sites (Fig. 1 and Table 2).

Our data suggest a significant proportion of the monarchs that originate in the Midwestern U.S. come from agricultural habitats. We estimate that cornfields and soybean fields together produce 78 times more monarchs than nonagricultural habitats in Iowa, and that cornfields produce 73 times more monarchs than nonagricultural habitats in Minnesota/Wisconsin. Nonagricultural habitats in the upper Midwest tend to produce more monarchs on a per area basis, but they are relatively rare, comprising <1% of the total potential monarch breeding habitat (Table 4), and thus are less

**Table 5. Phenological overlap (%) between first and second instars and corn anthesis**

Site	% Overlap			
	Minnesota/Wisconsin	Ontario	Maryland	Iowa
1	36	59	12	14
2	68	81	13	19
3	20	27	—*	11
4	44	66	57	21
5	30	75	0	9
Average	40	62	20	15

Estimates for Maryland and Ontario are likely overestimates because monitoring stopped before the end of the final generation.

\*No first or second instars were observed in Maryland Gannon site during the entire summer; it was not included in the average for that region.

important for overall monarch production. In Ontario, the landscape is more heterogeneous, with a greater proportion of nonagricultural habitats. Thus in Ontario, as least for the year in which the study was done, milkweeds in cornfields were not important contributors to monarch production.

Our productivity estimates are based on oviposition, but differences among habitats in the conversion of eggs into adults could affect monarch productivity. Larva survival data (Fig. 3 and Table 4) suggest that monarchs may suffer less early mortality in cornfields, at least in Ontario and Minnesota/Wisconsin. This difference may be a result of lower predator numbers in the less diverse cornfield habitat. However, by the end of the larva period, there were equal proportions of monarchs surviving in all habitats, thus our estimates use relative egg numbers.

These findings demonstrate that practices affecting milkweed densities in agricultural habitats are likely to have large impacts on monarch abundance. Farming practices such as tillage, herbicide use, and cropping choices may affect milkweed abundance and thus monarch numbers. Effects of tillage practices are unclear; Yenish *et al.* (20) found that fields in which conventional tillage is practiced have lower milkweed densities than fields undergoing conservation tillage practices, whereas Swanton *et al.* (21) found no consistent effect of tillage system on weed density, including milkweed, in corn. A possible reason for the low

**Table 4. Estimated milkweed density and monarch production over entire period monitored**

Region/habitat	No. milkweed observed	Eggs/milkweed	Milkweed density in study sites	Landscape milkweed density*	Relative monarch productivity <sup>†</sup>	Proportion of breeding habitat <sup>‡</sup>	Relative contribution of habitat <sup>§</sup>
Maryland							
Corn	25,566	0.0137	0.004/m <sup>2</sup>			<0.001	
Other agricultural	13,980	0.0378	0.003/m <sup>2</sup>			0.988	
Edge	13,425	0.0571	0.039/m <sup>2</sup>				
Nonagricultural	10,847	0.0360	0.027/m <sup>2</sup>			0.0123	
Minnesota/Wisconsin							
Corn	27,388	0.0612	0.285/m <sup>2</sup>	30 m <sup>2</sup> /hectare	0.65	0.124	73
Edge	5,493	0.0149	0.525/m <sup>2</sup>	102 m <sup>2</sup> /hectare	0.54		
Nonagricultural	13,257	0.0134	1.052/m <sup>2</sup>	212 m <sup>2</sup> /hectare	1	0.0011	1
Ontario							
Corn	12,125	0.0125	0.272/m <sup>2</sup>	0.008/m <sup>2</sup>	0.0081	0.302	0.055
Nonagricultural	12,097	0.0133	3.604/m <sup>2</sup>	0.924/m <sup>2</sup>	1	0.045	1
Iowa							
Corn	10,846	0.190		30 m <sup>2</sup> /hectare	0.24	0.436	45
Beans	9,124	0.208		16 m <sup>2</sup> /hectare	0.14	0.562	34
Edge	10,452	0.0313		102 m <sup>2</sup> /hectare	0.13		
Nonagricultural	4,337	0.114		212 m <sup>2</sup> /hectare	1	0.0023	1

\*Ontario estimates from random field survey in Ontario, and Iowa and Minnesota/Wisconsin from Hartzler and Buhler (10). Note that units are different in ref. 10; single stems or clumps of milkweed were assigned patch sizes of 1 m<sup>2</sup>.

<sup>†</sup>Productivity on a per area basis. Values obtained by multiplying columns 3 and 5, then relativizing to nonagricultural habitat.

<sup>‡</sup>U.S. values from Taylor and Shields (15). Ontario values from refs. 16 and 17.

<sup>§</sup>Relative contribution on a landscape basis calculated from egg densities and landscape data (contribution = relative productivity × proportion of breeding habitat), relativized to nonagricultural land.

densities of milkweed in Ontario and Maryland cornfields may be reduced tillage, which could favor clumped milkweed growth, thus making it easier to control (C. Swanton, personal communication). Additionally, more diverse cropping systems (as opposed to the corn-soybean rotation common in the upper midwestern U.S.) or herbicide use may lower milkweed densities in Maryland and Ontario cornfields.

Our conclusions require some caveats. More accurate estimates of the relative importance of different habitats will require extensive and random surveys of milkweed densities on a landscape scale, using consistent sampling methods; the methods used in Ontario for this study and by Hartzler and Buhler (10) were different, and these studies covered only a small portion of the monarch's breeding range. Our cornfields tended to have higher milkweed densities than random fields (Table 4), and this may have affected female oviposition behavior; however, the lack of a correlation between milkweed and monarch density suggests that nonrandom site selection may not have affected our results. Better information on land-use patterns will also add to the accuracy of estimates of relative productivity. Our observations were made during a single growing season; repeating the observations would allow us to generalize our results. Finally, calculations of relative productivity are reported without associated error terms and thus cannot be compared statistically. Fig. 1 illustrates the error associated with egg densities in each region; standard errors of milkweed densities in the field surveys in Ontario and Minnesota/Wisconsin ranged from 25 to 45% of the means. The Hartzler and Buhler (10) data on milkweed densities used for the upper Midwest were reported without error (15). Our goal was to estimate the relative importance of different habitat types, and our estimates suggest differences within and between regions ranging from one to two orders of magnitude (Table 4). This is the important finding, and more exact estimates will require additional data.

Despite these caveats, our study clearly indicates an assessment of risks imposed by Bt corn must consider pollen densities that fall on milkweed within cornfields. The small area of cornfield edge habitat, relative to the area of the fields themselves, and lower amounts of pollen that fall onto host plants outside the fields, make field margins less important in risk assessments.

**Probability of Exposure to Bt Corn Pollen.** In the northern study sites (Ontario and Minnesota/Wisconsin), the peak of the migratory monarch generation coincided with pollen shed in 2000, leading to the large phenological overlap shown in Table 5. This overlap was lower in Iowa and Maryland, where anthesis occurred before the peak of the migratory generation. The exposure this represents on a population-wide basis will depend on the proportion of larvae in cornfields and the proportion of fields planted in Bt corn. We can use Iowa, the region for which we have the most complete data, as

an illustrative example. If we assume that all monarchs from Iowa come from cornfields, soybean fields, or nonagricultural areas, the relative proportions of monarchs in the final column of Table 4 suggest that 56% of them originate in cornfields (45 is 56% of the total relative productivity of  $45 + 34 + 1$ ). If 15% of these are exposed to corn pollen (Table 5), and 35% of that pollen is from Bt corn (based on industry sources for the proportion of fields in Iowa planted to Bt varieties), then  $\approx 3\%$  of the monarchs emerging in Iowa over the course of a summer could be exposed to Bt pollen. This percentage will be higher further north, such as in Minnesota and Wisconsin where phenological overlap is  $\approx 3$  times greater (Table 5). Including later stadia in calculations would also increase the estimated overlap, but we did not do this to avoid counting individuals more than once.

Our measurement of corn anthesis occurred only at weekly intervals, thus the window of anthesis we measured in each field may be substantially longer or shorter than if our observation intervals had allowed for daily resolution. The time frame is consequential, because monarch life stages change on a scale of days rather than weeks. In addition, an accurate exposure assessment will require longer term data, because year to year temperature variations will affect corn and monarch phenology.

### Summary

Results presented here have two important implications. First, a portion of the monarch population is exposed to and probably consumes corn pollen that collects on milkweed plants growing in cornfields. Recent research suggests that the Bt corn hybrids most commonly planted produce levels of toxin in their pollen that are unlikely to have severe fitness consequences on monarchs (22–24), but our findings indicate the need to evaluate future transgenic hybrids on the basis of their protein toxicity and expression in pollen. Second, regardless of risks imposed by transgenic corn, changes in agricultural practices such as weed control or the use of foliar insecticides could have large impacts on monarchs by affecting milkweed density and condition, or monarch survival.

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## Catastrophic Winter Storm Mortality of Monarch Butterflies in Mexico during January 2002

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### INTRODUCTION

Monarchs form their overwintering colonies at high-altitude sites in the oyamel fir (*Abies religiosa*) forests in central Mexico. The butterflies are subject to freezing when northern cold fronts generate winter storms that impinge on high areas in the region. Since 1977 many storms have occurred, but their frequency, severity, and effects on the monarchs have not been systematically documented.

Calvert and coauthors (1983) reported on the effects of a prolonged frontal incursion that severely affected the Zapatero overwintering colony in the Sierra Chincua during January 1981. The storm system was accompanied by snow and sleet alternating with rain over 10 days. With subsequent clearing, the temperature in the butterfly colony dropped to  $-5^{\circ}\text{C}$ . They estimated that 418 monarchs were killed per square meter in the 0.65-ha colony, totaling 2.7 million dead monarchs.

Heavy mortality occurred again at the Sierra Herrada overwintering colony, located at 3164 m, in 1992 (Culotta 1992; L. Brower, pers. observ.). Prolonged cloudy, cold, and wet weather had occurred in the central overwintering region during January. A major cold front moved through during the early morning of 4 February, and by noon the whole of central Mexico was spectacularly clear. At 12:30 AM, the temperature was  $2.5^{\circ}\text{C}$  and the area was still very wet. Dead monarchs littered the ground and the colony area had been reduced by 90% (W. H. Calvert, pers. comm.). Brower and Calvert (unpub.)

estimated that more than 80% of the monarchs had been killed. Heavy mortality had also occurred in the Sierra Chincua and Sierra Campanario colonies (C. Gottfried and Rosario guides, pers. comm.). We hypothesized that the butterflies had been killed by the combination of wetting and the subsequent clearing that results in extreme radiant heat loss to the cloudless sky. Anderson and Brower (1993, 1996) tested this hypothesis at a field laboratory in the Sierra Chincua. They measured the cumulative mortality that occurred as the body temperatures of individual monarchs were experimentally lowered to  $15^{\circ}\text{C}$  below freezing. The study determined that dry butterflies have a modicum of cryoprotection and can super-cool, but by  $-8.1^{\circ}\text{C}$ , 50% had frozen to death and by  $-15^{\circ}\text{C}$ , all were killed. When misted, they lost much of their natural cryoprotection: 50% died as their body temperatures dropped to only  $-4.4^{\circ}\text{C}$ , and by  $-7.8^{\circ}\text{C}$ , all were dead. Larsen and Lee (1994) showed that wetting also accelerates the freezing process: monarchs held at  $-4^{\circ}\text{C}$  froze within 3 hours when wet, whereas dry individuals survived the same treatment for the 24-h duration of the experiment. These studies are definitive: wetting of butterflies prior to the short-term temperature plunges that follow winter storms in the overwintering region greatly increases the percentage of monarchs that will freeze to death.

Continuing microclimatic research at the overwintering sites in Mexico demonstrated that forest thinning exacerbates the monarchs' risk of freezing (Calvert and Brower 1981; Calvert and Cohen 1983;



Calvert et al. 1982, 1983, 1984, 1986; Alonso-Mejía et al. 1992; Anderson and Brower 1993). Removal of trees within and adjacent to the overwintering colonies subjects monarch clusters to increased wetting and their individual body temperatures drop below the ambient temperature when it is clear. This happens because body heat is lost by radiation through gaps in the forest canopy to the colder sky (Anderson and Brower 1996).

Recognition during the 1980s that forest thinning in and near the overwintering sites posed a severe threat to monarchs resulted in the designation of their migration and overwintering biology as an endangered biological phenomenon (Wells et al. 1983; Brower and Malcolm 1991). Brower (1996b, 1999a, 1999b) synthesized the evidence that forest thinning and the overwintering of monarchs are incompatible and called for more effective protection of the oyamel-pine ecosystem. A geographic information system (GIS) analysis of deforestation between 1971 and 1999 revealed that 44% of the high-quality forest has been degraded and fragmented in the past 28 years, and that the rate of cutting is accelerating (Brower et al. 2002).

Misunderstandings about monarch biology, combined with insufficient data, have resulted in erroneous reports in the press and a general public confusion over rates and causes of winter mortality. In December 1995, for example, an early winter storm dropped 31 cm of snow on the Sierra Chincua. Clearing was accompanied by heavy winds that dislodged millions of butterflies from their clusters. It was reported in the press that up to 35% of the colony had been killed (e.g., Preston 1996). In fact, because little rain accompanied the storm, its impact was minimal. Most of the butterflies recovered and the mortality estimate was changed to 5% to 7% (Brower 1996a). This and a spate of other press reports resulted in accusations of exaggeration of the effects of logging (e.g., Huriash 1996).

Once again in January 2001, lack of understanding of the biology of monarch overwintering generated severe controversy about what had killed tens of thousands of monarchs at the San Andrés overwintering colony. Iniquitous pesticide spraying was implicated as the cause by concerned visitors to the area and then widely reported in the press and popular monarch literature (Aridjis 2001; Fullerton 2001; Marriott 2001). The pesticide hypothesis received no support from independent chemical

analyses done for the Mexican federal environmental enforcement agency (PROFEPA 2001). Brower (2001) concluded that the most likely explanation was a winter storm that soaked and then froze thousands of monarchs, followed by the colony's partial recovery and subsequent movement to a nearby location.

On 12 to 14 January 2002, a heavy winter storm occurred in the monarch overwintering region in central Mexico. We describe the storm and the impact it had on four different overwintering colonies (Conejos and Rosario [Sierra Campanario], Zapatero [Sierra Chincua] and Capulin [Cerro Pelón]) and discuss conservation implications.

## METHODS

### Locations, altitudes, and sizes of the colonies

Figure 15.1 illustrates the locations of the mountains on which monarch colonies are known to form. During January and March 2002 we used an Eagle Explorer global positioning device to determine the coordinates of overwintering sites, and a Thommen altimeter to determine altitudes, referenced against 1:50,000 topographic maps (Anonymous 1974, 1987). One of us (E. García-Serrano) measured colony areas on 13 to 20 December 2001 (table 20.1). The Zapatero and Rosario colonies have formed nearly every year since 1976 and 1980, respectively, when they were first reported (Urquhart 1976; Calvert and Brower 1986; Bojórquez-Tapia et al. 2003).

### Weather observations

To reconstruct the storm climatology and the likely minimum temperatures reached in the Zapatero colony, we pieced together three sources of information. First, during and after the storm, one of us (D. R. Kust) recorded temperatures, rainfall, and qualitative weather descriptions in an open courtyard in the town of Angangueo, in a grove of low-growing juniper trees next to Chincua Station, and along the 4-km dirt road leading from the station into the Zapatero colony. Temperature was measured with a Springfield Precise Digital thermometer.

Second, hourly satellite weather images (GOES-EAST 2002) posted on the Internet by David Dempsey showed the genesis and movements of an



Table 20.1. Approximate center locations and altitudes of the overwintering colonies and weather stations

Location	Area (ha)	Massif	Latitude (N)	Longitude (W)	Altitude (m)
Rosario colony	0.10 <sup>a</sup>	Campanario	19°35'56"	100°15'55"	3200
Conejos colony	2.59 <sup>a</sup>	Campanario	19°35'45.2"	100°15'50.0"	3277
Zapatero colony	2.96	Chincua	19°40'41.3"	100°18'25.6"	3206
Capulin colony	0.98	Pelón	19°23'33.7"	100°16'44.2"	2896
Campamento colony		Pelón	19°23'48"	100°16'52"	2752
Chincua station		Chincua	19°39'42.8"	100°16'4.0"	3182
Town of Angangueo		Chincua	19°37'30.0"	100°16'46.0"	2630

<sup>a</sup> Areas of the closely adjacent Rosario and Conejos colonies are combined in the analyses as 2.69 ha. Also shown are pre-storm areas of the four monarch overwintering colonies measured in December 2001. Zapatero was measured on 13 December; Rosario and Conejos, on 14 December; and Capulin, on 20 December.

extensive storm system that passed across the overwintering region from 11 to 14 January. We examined 72 hourly visible and infrared images and selected six of the latter to construct figure 20.1.

Third, we compared the daily minimum temperature measurements made by D. Kust during and immediately after the storm with temperatures measured in March, and estimated the likely minimum temperatures in the Zapatero colony on the three clear mornings following the storm. We recorded the air temperature for 15 days (5 to 19 March 2002) at 15-min intervals using four pre-calibrated Hobo XLT electronic thermometers. One Hobo was set in the courtyard in Angangueo, two were suspended in the center of a standard weather box at the Chincua Station (both recordings were virtually identical), and one was set in what had been the center of the Zapatero colony at the time of the storm. To avoid vandalism, we placed the latter sensor inconspicuously in contact with the surface bark of an oyamel fir. Previous research in the Sierra Chincua indicated that the daily minimum ambient temperature is at least 1°C lower than the minimum temperature recorded on the bark surface (Brown, Lear, García-Serrano, pers. observ.). A difference in 1° can have a substantial impact on the probability of wet monarchs freezing (Anderson and Brower 1996).

Measuring monarch mortality

Initial qualitative observations: 1 to 4 days after the storm

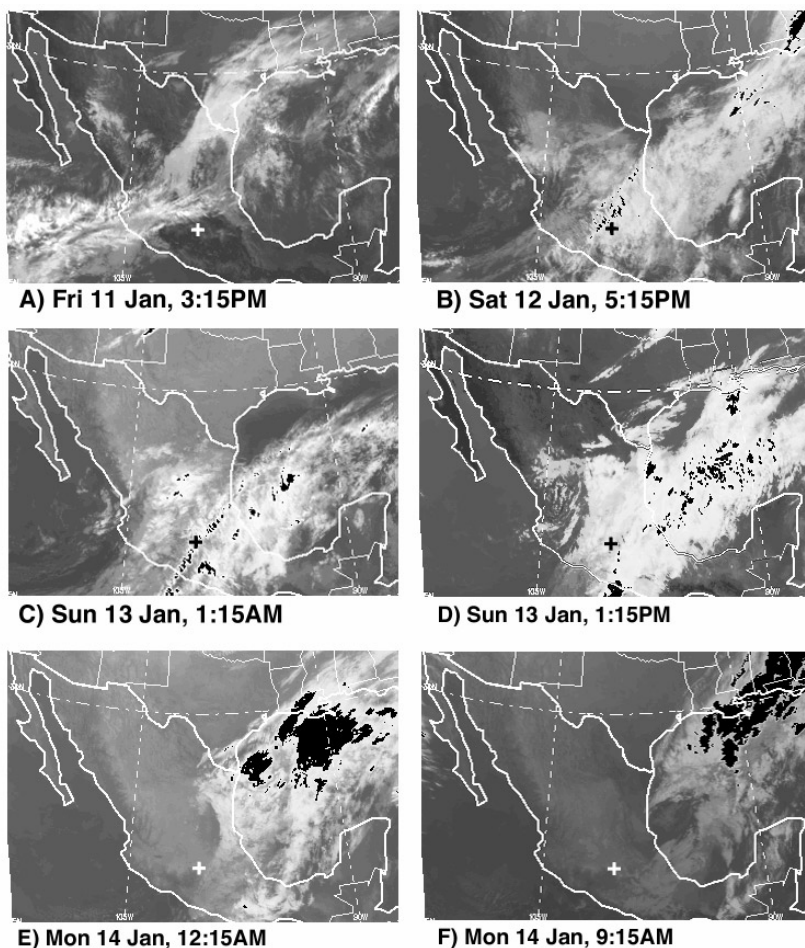
D. Kust visited the Zapatero colony immediately after the storm on 14 and 16 January and the Rosario and Conejos colonies on 18 January. During these

visits, he qualitatively assessed the level of mortality caused by the storm.

Quantitative mortality estimates: 7 to 8 days after the storm

We (except for E. García-Serrano and E. Rendón-Salinas) visited the Rosario and Conejos colonies on 21 January and the Zapatero colony on 22 January. At Rosario, we collected three samples of butterflies in gallon-size plastic bags from the ground beneath the roost trees, about 5 m off the path and a few meters apart from each other. To ascertain their status, we emptied the samples onto the ground in a nearby open field and spread the butterflies out to warm for several minutes in the bright sun. The ambient shade temperature was 22°C, well above flight threshold. Any individuals showing movement of their appendages were grasped and tossed into the air. As in the study by Calvert and colleagues (1983), those that flew normally were categorized as alive; those that flew abnormally or plummeted to the ground were categorized as moribund (effectively dead); and those that showed no signs of movement of their legs, wings, antennae, or proboscis were categorized as dead. We tallied and sexed the butterflies in each category between 2:55 and 3:40 PM, during which the weather was clear with occasional light clouds. Immediately after this, we moved to the Conejos site and followed the same procedure to categorize butterfly condition. We completed data collection before 5:45 PM, while it was still sunny.

The following day, we sampled six 25 × 25-cm (625-cm<sup>2</sup>) plots in the southwestern quadrant of the Zapatero colony. We finished collecting our samples too late to warm them in the sun, so we punched holes in the plastic bags and took them to



**Figure 20.1.** Six infrared satellite images generated for 11 to 14 January 2002 document a major North American winter storm system that penetrated Mexico. The white or black plus signs denote the center of the Sierra Chincua sanctuary. The GOES infrared image numbers from (a) to (f) are 02011-121, -223, -307, -319, -406, and -415, respectively, and have been converted to central standard time (GOES EAST 2002). The storm system moved from the Pacific Ocean northeastwardly across Mexico while the precipitation line (blackened areas) swept across the overwintering region with a southeasterly trajectory. In agreement with these satellite data, our ground observations indicated that heavy rain occurred on Saturday and Sunday, followed by extreme clearing beginning late on Sunday night.

Angangueo. We transferred each sample into a pillowcase, spread the monarchs out as thinly as possible, and left them overnight on an open, cold patio floor, next to a thick wall to prevent freezing by radiational cooling during the night. The next day the weather was again clear and we tallied the monarchs from 10:10 AM to 4:30 PM. We split each sample into several separate pillowcases and hung the cases on a clothesline in the sun for about 15 min. This enabled us to separate the dead from the living because the latter crawled up and could be seen in silhouette. We then tossed the survivors individually into the sunlit patio and recorded their condition as

just described. We emptied the remaining monarchs onto a table and discarded those that had long been dead or killed by birds, categorized according to Fink and Brower (1981). The total of long-dead and bird-killed monarchs provides an estimate of the cumulative mortality caused by factors other than the storm.

#### Quantitative mortality estimates: 16 days after the storm

D. Kust and Rendón Salinas returned to Zapatero on 30 January, 16 days after the initial freeze. They established a reference line through the center of

what, in December, had been an elliptical colony that embraced the dry streambed. They then marked two 140-m sampling lines parallel to and 20 m to the left and right of the reference line. At 10-m intervals they alternately walked perpendicularly for 5 m to the left and then 5 m to the right off of the main line, establishing 14 sampling positions on one line and 15 on the other. The position of the samples was at the end of each 5-m arm. They followed this procedure to obtain 29 additional samples along two 150-m transects through the Conejos colony on 31 January.

At each sampling position monarchs were gathered from 20 × 20-cm squares (0.04 m<sup>2</sup>) and put into labeled plastic bags. After removing the butterflies from each sample square, D. Kust and Rendón-Salinas measured the depth (cm) of the stacked butterflies on one edge of the cleared sample area. In assessing butterfly condition, they discarded long-dead individuals and categorized the rest as alive, moribund, dead with evidence of bird predation, or dead from other cause (attributed to the storm).

tion, or dead from other cause (attributed to the storm).

RESULTS

Weather observations

Local observations

From 2 to 11 January 2002, the region was clear with few clouds and no precipitation (table 20.2). On 12 January a storm front moved in from the southwest and, as observed in Angangueo, was accompanied by alternating periods of light and heavy rain. Rain continued intermittently through the night and the next day until 10:00 PM, accumulating to a total of 5.8 cm. No snow accumulated in Angangueo, but by midday on 13 January at Chincua Station the rain had turned to snow. Clearing occurred by 11:00 PM on 13 January and it remained clear through 17 January (table 20.2).

Table 20.2. Weather observations

Date	Time	Location	Temperature (°C)	Remarks
3–10 Jan		Entire region		Generally clear with few clouds, no precipitation
11 Jan	7:00 AM	Angangueo	5.4	Clear all day
12 Jan		Angangueo		Storm front arrives, continuous light rain during day, intermittently harder all evening
13 Jan	7:00 AM	Angangueo Chincua Station	5.6	Rain continues all day, intermittently heavy
				Rain turns to snow in afternoon, accumulating at high elevations throughout the area
	6:30 PM	Angangueo	7.5	Rain continues; cold front arrives with increasing wind
	10:00	Angangueo		Rain ends
	11:00	Angangueo	3.5	Clear, total rain accumulation = 5.8 cm
14 Jan	7:00 AM	Angangueo	−0.2	Clear, thin ice layer in bucket
	7:00	Zapatero colony	−4.4	(Temperature estimated)
	10:30	Angangueo	7.5	Clear
	12:00 PM	Chincua Station		Clear; 5 cm of snow on ground in shaded areas, people making snowmen
	12:00 PM	Road to Zapatero		Ground extremely wet, with snow and ice, refrozen crunchy snow in shaded areas
15 Jan	7:30 AM	Angangueo	1.4	Clear
	7:30	Zapatero colony	−2.8	(Temperature estimated)
	12:00 PM	Chincua Station		Snow remains and puddles frozen in shaded areas, water and mud in road where sun hit
16 Jan	7:30 AM	Angangueo	5.7	Clear
	7:30	Zapatero colony	1.5	(Temperature estimated)
	8:00	Chincua Station	−4.2	Clear; ambient shade temperature measured in juniper grove
	8:30	Road into colony		Heavy rime ice and frost on vegetation, many puddles frozen sufficiently to walk on
	1:00 PM	Zapatero colony	13.8	Ambient shade temperature in colony; snow melt running down road adjacent to colony
17 Jan	7:00 AM	Angangueo	7.1	Clear
	7:00	Zapatero colony	2.9	(Temperature estimated)

On 14 January, about 5 cm of snow covered the ground at Chincua Station and along the road to the Zapatero colony (table 20.2). Based on a snow-to-rain conversion factor of 4:1 for wet snow (Anonymous 2002), 5 cm of snow would be equivalent to about 1.25 cm of rain. Assuming that the Zapatero colony area received the same amount of precipitation as Anganguero (5.8 cm), the butterfly colony would have received 4.55 cm of rain in addition to the 5.0 cm of snow. Anganguero and Zapatero are located only 6 km apart on the Chincua-Campanario massifs, and the colony is 580 m higher in altitude. Because of the adiabatic effects of the wet air mass being forced up over the Sierra Chincua, more precipitation than this probably fell on the butterfly colony area.

At 8:00 AM on 16 January the temperature at Chincua Station was  $-4.2^{\circ}\text{C}$  with large puddles frozen sufficiently to walk on. The ground and surrounding vegetation (grass, herbs, bushes, and trees) were frosted with rime ice. Since rime forms by deposition of super-cooled water drops floating in a driving fog that crystallizes on surfaces (Geiger 1950), its presence was another indication of how wet the area had become.

Satellite images support D. Kust's local weather observations and indicate that this storm system affected all the known monarch overwintering massifs (see figure 20.1).

#### Temperature estimates for the Zapatero colony

The difference between the minimum temperatures recorded on 16 January at Anganguero and the Chincua Station was  $9.9^{\circ}\text{C}$  (table 20.2). The average difference of the daily minimum temperatures in Anganguero and Chincua Station measured for 15 days in March was  $9.2^{\circ}\text{C}$  (Anganguero mean =  $10.08^{\circ}\text{C}$ , Chincua mean =  $0.90^{\circ}\text{C}$ ). The closeness of these two values suggests that we can use the measured temperature differences in March to estimate the Zapatero colony temperatures after the storm from measurements taken in Anganguero. The average minimum temperatures in March were  $10.1^{\circ}\text{C}$  in Anganguero and  $5.9^{\circ}\text{C}$  in the Zapatero colony, a difference of  $4.2^{\circ}\text{C}$ . Subtracting  $4.2^{\circ}\text{C}$  from the minimum temperatures measured in Anganguero on 14 to 17 January, we estimated the minimum temperatures attained on these four clear mornings following the storm in the Zapatero colony (table 20.2). If the ambient temperature in Zapatero was

on average  $1^{\circ}$  colder than the oyamel bark temperature as described earlier, then these minima would be reduced accordingly.

Even though the Zapatero colony and Chincua Station are at nearly the same altitude, the average minimum temperature inside the colony in March was  $5.0^{\circ}\text{C}$  warmer than at the station (Zapatero =  $5.86^{\circ}\text{C}$ , Chincua Station =  $0.90^{\circ}\text{C}$ ). This is likely because the station weather box was located within a large clearing, whereas the intact forest above the butterfly colony acted as a microclimatic blanket.

#### Monarch mortality

##### Initial qualitative observations (1 to 4 days after the storm)

D. Kust and one of the Chincua Station guides were the first to arrive at the Zapatero colony after the storm, at 1:30 PM on 14 January. They walked only a meter into the colony to avoid damaging the many downed and very wet monarchs. Binocular viewing indicated little movement of live butterflies in the colony, and the clusters on the oyamel branches seemed still intact. There were no large accumulations of monarchs on the ground, but D. Kust observed individual monarchs plummeting to the forest floor with regularity.

He returned to the Zapatero colony on 16 January and reported that many monarchs were now on the tops of the understory shrub layer. The scene seemed less severe than on Monday, though many monarchs continued to fall from the trees. He noted that the shade temperature by 1:00 PM had risen to  $13.8^{\circ}\text{C}$ , less than  $2^{\circ}$  below flight threshold (Masters et al. 1988). Thousands of monarchs were basking in the sun on tree boughs and understory vegetation and drinking water in sun-lit puddle areas. Later in the afternoon many were airborne, and both the trees and the ground were festooned with butterflies. A temperature mosaic in the colony was indicated by the presence of snow and ice patches. It appeared that fewer monarchs were falling from the trees than 2 days previously. At this time, D. Kust was not certain whether the accumulated monarchs on the ground were dead.

On 18 January, D. Kust arrived at the Rosario colony at 10:00 AM. The guards had swept dead monarchs from the main tourist path into piles and had blocked several other paths through the colony. The logging road through the Conejos colony was

covered with thick layers of immobile monarchs. In both colonies there was little butterfly movement, and it appeared that many of the intact clusters that D. Kust had seen on 11 January prior to the storm were no longer present on the tree branches. At this time, he estimated mortality to be highest in Conejos, intermediate in Rosario, and least in Zapatero, and reported his findings on Journey North (2002).

#### Quantitative mortality estimates: 7 to 8 days after the storm

**Rosario colony.** When we arrived at Rosario on 21 January, the weather, except for a general haze, was clear and the ambient shade temperature in the oyamel forest at 2:30 PM was 15°C, very close to flight threshold. The scene was unlike anything one of us (L. Brower) had witnessed over 25 seasons; had any monarchs on the ground been alive, they would have been fluttering and crawling onto the understory vegetation and tree trunks. Instead, deep layers of motionless butterflies littered the forest floor. D. Kust observed that many more had accumulated on the ground since 16 January. Of the 745 butterflies we counted in three separate samples, 1% were alive, 1% were moribund, and 98% were dead. There was a significant excess of males (64%).

**Conejos colony.** On reaching Conejos, we observed masses of dead monarchs on the logging road through the center of the colony. A few meters into the north central side, accumulations on the ground beneath the formerly monarch-laden oyamels ranged from 0 to over 20 cm deep. Numerous butterflies lay motionless on the tree boughs while many of the oyamels had intact, seemingly normal clusters. We shook several smaller trees and determined that both the motionless butterflies and those in the clusters had frozen in situ. The understory vegetation showed considerable frost damage, especially the *Senecio angulifolius* D.C. bushes, the leaves of which were blackened and shriveled. A 3-m-high *Cupressus benthamii* var. *lindleyi* Kotsch tree in the understory had many quiescent monarchs hanging on its boughs. We picked off 100 and gently blew on them in our cupped hands. If they had still been healthy, this would have warmed them sufficiently to fly. All were dead. We collected three (nonrandom) separate ground samples from measured areas. Of

965 monarchs sampled, 5 were moribund, and the rest were dead. The calculated numbers of dead monarchs/m<sup>2</sup> were 4297, 4502, and 2290. The sex ratio was approximately even.

**Zapatero colony.** As we approached Zapatero colony via a road from the Llanos de los Villalobos along the north face of the Sierra Chincua, we noticed severe frost damage on the understory *S. angulifolius* bushes in thinned areas beneath the oyamels. Most were browned except for their lowermost leaves. They still had snow on them and the ground was very wet. The colony was located at the head of El Zapatero watershed in virtually the same location as in several previous years (Calvert and Brower 1986).

We saw similar mortality patterns as in Conejos, with layers of monarchs covering both the forest floor and the adjacent logging road. Heterogeneity in the depth of the accumulations was evident: where tree branches extended from the oyamels, many more monarchs were on the ground than beneath the many forest gaps.

The leaves of the 1.5-m *S. angulifolius* bushes in the colony beneath the oyamels on the southwestern side of the creek bed were still mostly green, compared to the severe frosting on the ridge about 160 m above the colony. Many monarchs clung to the upper leaves of these bushes. We cupped several in our hands, gently blew ten breaths on them, and then tossed them into the air. Twelve (8%) of 146 monarchs flew normally while the rest were moribund. We collected six ground samples from measured areas, totaling 4288 monarchs. In five of the six samples, only 1.2% to 2% of the butterflies were alive, but in the deepest (20-cm) sample, many monarchs (38%) were buried alive. The butterflies that covered them probably kept these individuals alive, but the weight of the tightly packed dead butterflies on top of them would likely have caused their eventual death. From these six samples we estimated an average of 9854 dead monarchs/m<sup>2</sup> (standard deviation [SD] = 7540, range = 1456 to 18,112). These estimates of mortality were 3 to 43 times larger than the 418 estimated killed per square meter during the 1981 storm (Calvert et al. 1983).

#### Quantitative mortality estimates: 16 days after the storm

Preliminary data from the Rosario, Conejos, and Zapatero colonies indicated high sample variability



**Table 20.3. Monarch conditions in 29 random samples made in the Zapatero and Conejos colonies**

Colony	Sample means (SD) and ranges for 0.04-m <sup>2</sup> plots					Estimated no. dead / m <sup>2</sup>
	No. dead	No. moribund	No. live	No. killed by birds	Depth (cm)	
Zapatero ( <i>n</i> = 3136)	99.4 (89.1) 0-337	5.8 (7.6) 0-35	1.5 (3.7) 0-16	1.5 (1.4) 0-5	3.8 (2.9) 0-9	2628
Conejos ( <i>n</i> = 8489)	289.8 (504.5) 1-2347	0.4 (1.3) 0-7	0.03 (0.19) 0-1	2.6 (6.4) 0-32	6.5 (8.5) 0-33	7253

*Note:* The estimated number of dead monarchs per square meter includes dead and moribund monarchs [(99.4 + 5.8) × 25], [(289.8 + 0.4) × 25]. SD, standard deviation.

**Table 20.4. Mortality estimates in the Zapatero and Conejos colonies**

Colony	Colony size before storm (ha)	Colony size after storm (ha)	% of colony eliminated	No. of monarchs killed / m <sup>2</sup>	No. of monarchs killed / ha (millions)	Total no. of monarchs killed (millions)
Zapatero	2.96	0.76	74.3%	2628	26.3	77.8
Conejos	2.69	0.54	80.1%	7253	72.5	195.1

and heterogeneity in the depth of accumulations of dead monarchs. We therefore deemed it critical to obtain a larger number of samples collected systematically from within the pre-storm colony boundaries. This was facilitated by García-Serrano having marked the colony borders in December.

**Zapatero colony.** By 24 January, the surviving monarchs had deserted the area in which the mortality had occurred. Dropping about 35 m in altitude, they moved slightly down the dry streambed and re-formed a smaller (0.76-ha) colony on the trees on both sides of the arroyo.

Table 20.3 summarizes the conditions of the butterflies in the 29 0.05 m<sup>2</sup> samples. Of 3136 butterflies collected, 44 (1.4%) were alive, 167 (5.3%) were moribund, 43 (1.4%) were preyed on, and 2882 (91.9%) were dead from other causes, assumed to be storm-related mortality (mean numbers in each category are illustrated in table 20.3). There was a small deficiency of females in all categories. The average depth of accumulated dead butterflies was 3.8 cm. Using the mean number of dead and moribund monarchs per sampling plot, we calculated the mean number of dead and moribund monarchs per square meter (number/0.04-m<sup>2</sup> plot × 25 = number/m<sup>2</sup>). The average of 2628 dead or dying monarchs per square meter is more than six times the mortality

estimated during the 1981 storm (Calvert et al. 1983). By multiplying the mean number of dead or dying monarchs per square meter by the pre-storm colony size, we estimated the total mortality at the Zapatero colony to be 77.8 million monarchs (table 20.4). Because the post-storm cluster densities were substantially less than they had been prior to the storm, we feel confident that this is a conservative estimate of the mortality caused by this storm.

**Conejos colony.** As in Zapatero, the surviving butterflies deserted the original colony area. They moved slightly up the slope where they formed a distinct new colony. D. Kust and Rendón-Salinas revisited the area on 31 January and concluded that the colony architecture was so disturbed and fragmented that its post-storm area could not be accurately measured. However, García-Serrano had estimated that 942 trees were occupied by butterfly clusters on 14 December, and D. Kust and Rendón-Salinas now counted 187 trees in the recovery colony, an 80.1% reduction. We used this percentage to estimate the reduction in colony size from the pre-storm value of 2.69 ha to a post-storm size of 0.535 ha (table 20.4).

Of the 8489 monarchs collected in 29 samples at Conejos, 1 was alive, 11 were moribund, 74 were preyed on, and 8412 (99%) were dead (see table

20.3). Sex ratios were nearly even. The average depth of accumulated dead butterflies was 6.5 cm (figure 20.2). We estimated an average of 7253 dead or dying monarchs per square meter at the Conejos colony, yielding an overall estimate of 195.1 million monarchs killed. As was true for the Zapatero colony after the storm, visual inspection indicated that the post-storm clusters were substantially less dense. We therefore are again confident that our data provide a conservative estimate of the mortality caused by this storm.

#### Additional transect samples

García-Serrano had also sampled the Zapatero and Conejos colonies to estimate mortality, and his methods yielded substantially different estimates than the sampling by D. Kust and Rendón Salinas just described. He had collected 57 samples along three transects through Zapatero on 23 January and 59 samples along three transects through Conejos on 24 January. His collecting and sampling methods differed in three important ways: (1) they were collected 9 and 10 days after the 14 January freeze, whereas D. Kust and Rendón-Salinas collected samples 16 and 17 days after the freeze; (2) García-Serrano collected monarchs from 1.0-m<sup>2</sup> areas, while D. Kust and Rendón-Salinas's were from 0.04-m<sup>2</sup> areas; and (3) García-Serrano stored his samples in numbered plastic bags at the Sierra Chincua research station and did not himself tally or supervise the tallying of his samples. It was not possible to obtain counts of all of his 116 samples, but in early February, D. Kust and Rendón Salinas tallied 15 from each colony.

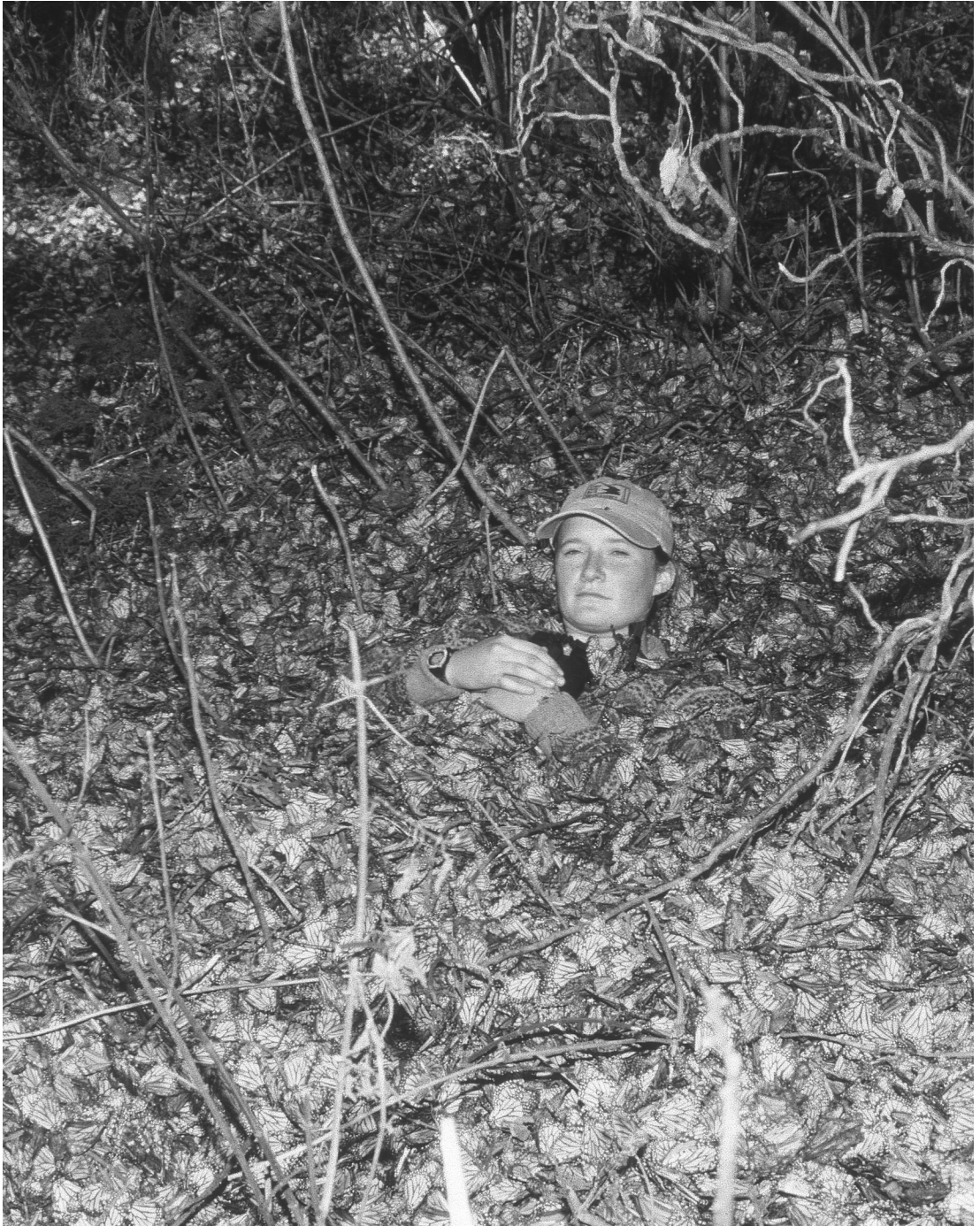
They estimated 749 and 245 dead monarchs/m<sup>2</sup> at the Conejos and Zapatero colonies, respectively. These two estimates were 9.7 and 10.7 times less than those from the samples collected later (7253/749 and 2628/245). The similar ratios suggest a biological basis for the discrepancy. Monarchs continued to fall from the trees and accumulate on the ground for several weeks after a similar previous freezing event (Calvert et al. 1983), and D. Kust observed the same phenomenon occurring in both colonies, with dead monarchs still falling out of the Zapatero trees on 30 January. Four of us (García-Serrano, Rendón Salinas, D. Kust, and L. Brower) discussed the apparent discrepancy and agreed that the differences between our estimates had likely resulted from continued accumulation of dead

monarchs on the ground during the time between the two sampling periods.

**Comparison of mortality in the Conejos and Zapatero colonies.** Although the 2.76-fold difference in mean densities of dead and moribund monarchs between Conejos and Zapatero was not statistically significant (Mann-Whitney  $Z = 0.91$ ,  $p = 0.363$ ), several lines of evidence led us to conclude that Conejos was harder hit. First, the largest number of dead monarchs per sample in Zapatero was 337. In contrast, six samples in Conejos had more than 450 dead monarchs, including one with 2347, more than six times the maximum in Zapatero (see table 20.3). Second, the mean depth of accumulations in Conejos was 1.72 times that of Zapatero, and the maximum depth recorded in Conejos was 33 cm compared to 9 cm in Zapatero (see table 20.3). However, the high variance again precluded showing a significant difference in the depth of the samples ( $t = 1.64$ ,  $df = 56$ ,  $p = 0.11$ ). Third, in all samples from Zapatero combined (from the 21 and 30 to 31 January collection dates), 643 of 7424 butterflies were alive. In contrast, in the Conejos samples, only 1 of the 9454 monarchs was alive. The numbers of moribund butterflies showed the same pattern. Fourth, the leaves on the *Senecio* understory in Zapatero showed only minor damage, whereas in Conejos the apical stems and leaves were bent over, shriveled, and blackened and had entombed many monarchs. Finally, the separate set of samples gathered in the two colonies by García-Serrano indicated a similar magnitude of difference (3.06 times as many dead and moribund monarchs in Conejos as in Zapatero).

#### Qualitative observations: 7 weeks after the storm

**Rosario, Conejos, and Zapatero colonies.** We revisited the Rosario and Conejos colonies on 5 March and the Zapatero colony on 4 and 6 March and confirmed that the surviving butterflies had deserted their original sites and re-formed colonies in locations within approximately 0.2 to 1.0 km of the positions they occupied during the January storm. As we describe later, the Pelón colony responded similarly. It therefore appears that after suffering a major mortality event, monarchs move and reestablish their overwintering colony some distance from the accumulated dead butterflies.



**Figure 20.2.** Chris Kiesel is shown covered in dead monarchs in the Conejos overwintering colony on Sierra Campanario. This exemplifies the severe mortality caused by the January 2002 storm. We estimated that 195 million monarchs were killed in this colony (see table 20.4). Photo by L. P. Brower, 5 March 2002.



The general appearances of the surviving remnant colonies were very different from what they had been before the storm. In addition to occupying smaller areas and fewer trees, bough clusters on individual trees were fewer, smaller, and more dispersed. In all sites except Zapatero, the new locations of the colonies were in more thinned areas of the forest and therefore more exposed to the sky.

Colonies typically move down their arroyo as the season progresses (Calvert and Brower 1986). In contrast, the monarchs in Conejos moved upward into a section of the oyamel forest that had been heavily thinned in the late 1980s (L. Brower, pers. observ.). In Rosario, they moved laterally and slightly up into the oyamel forest that has been progressively thinned. Finally, as we describe later, in the heavily damaged forest on Pelón, the El Capulin colony that had formed at a perilously low altitude moved even farther down the arroyo into a largely deciduous forest.

These observations suggest that normal colony movements to lower altitudes in the watershed were disrupted. Ending up in more, rather than less, exposed areas of the forests further jeopardizes the monarchs because of the potential for sequential winter storms.

Our return in March to the pre-storm colony areas confirmed our observations on the vastness of the mortality. Many monarchs that had accumulated on the oyamel boughs still had not fallen to the ground. The heavy and compressed butterfly packs were now molding and decomposing. The large stores of lipids in the butterfly abdomens were leaking out and penetrating the wings, turning them shiny and black. The air was scented with a disturbing mixture of ammonia and sweetness.

**Cerro Pelón.** Manos-Jones and Taylor (2002) reported that an overwintering colony on Cerro Pelón had not been impacted by the January storm. This position was also strongly espoused by a prominent ecotourism leader, Astrid Fritsch Jordan, who also maintained that the storm had missed the Herrada colony (pers. comm.). The Pelón and Herrada massifs are about 20 to 60 km southeast of the Chincua and Campanario massifs (see figure 15.1). Brower was skeptical because of a report that the storm had damaged the original Herrada colony, and that the surviving butterflies had moved and re-

formed a colony at lower altitude (J. Honey-Rosés, pers. comm.).

To resolve the issue, we visited the El Capulin colony at the Cerro Pelón overwintering area on 3 March via the small village of Macheros, located at 2490 m on the southwest side of Cerro Pelón. The weather was brilliantly clear and the ambient temperature well above the monarch flight threshold. At 2740 m we encountered several thousand monarchs roosting in moderate-sized pine trees and flying against the azure sky. On reaching the 0.98-ha monarch colony that García-Serrano had measured in December (see table 20.1), we observed that the devastation caused by the storm seemed at least as severe as in Conejos. We saw no live monarchs within the bounds of the now deserted colony area, dead butterflies littered the ground, thousands were buried in layers of dry pine needles, huge piles had accumulated beneath oyamel and pine boughs, and many of these boughs cradled monarchs that had frozen in situ.

The center of the deserted Capulin colony was at 2896 m, nearly 300 m lower in altitude than the current and past Rosario, Conejos, and Campanario colonies (see table 20.1). We attribute this anomalous altitude (see also Calvert and Brower 1996) to the severe habitat deterioration. We did not quantify the mortality, but it was clear that reports of no storm impact on the Cerro Pelón monarchs were incorrect. Leaving the colony, we walked 0.5 km northwest into the arroyo that drains the entire watershed (Las Canoas). Here we encountered several people viewing another colony of live monarchs called the Campamento colony (see table 20.1). We learned that Campamento can be accessed via two routes: the one we had taken on the southwest side of Cerro Pelón, or an alternative route on the northwest side of Cerro Pelón.

We concluded that the Campamento colony was actually a small surviving remnant of the Capulin colony that had moved several hundred meters down the arroyo after the January storm. As in the case of the Conejos and Zapatero colonies, the monarchs that survived the January storm deserted the Capulin colony area and formed another colony in the same arroyo at 2752 m, dropping about 0.5 km and 145 m in altitude below the original colony. Because of the near absence of oyamels and pines in the new site, the monarchs had reestablished most of their clusters on the moderate-sized oak trees growing along the sides of the arroyo. These clusters

were less structured and much less dense than usual, probably owing to both the deciduous tree architecture and the diminished numbers of monarchs now more scattered within the colony area.

We are confident that Campamento was not a distinct colony that had formed the previous fall because García-Serrano had passed through the Campamento site in December and had observed no monarchs. Local residents confirmed that this was a newly formed site, and we observed very few dead butterflies on the ground beneath the new colony, further evidence that it had formed after the storm.

We were impressed by the severe dryness of the Pelón area compared to the colony locations on the Sierra Campanario and Sierra Chincua. Although one small dammed water pool was present slightly above the Campamento colony, we found no springs, seeps, or running water as we descended the arroyo until we encountered plastic pipes capturing spring water at 2646 m, about the same altitude as Angangué (see table 20.1). The extensive deforestation appears to have disrupted the hydrology of the entire Canoas watershed on this part of Cerro Pelón. Thus monarchs, heavily impacted by the January storm in the exposed remnant oyamel pine forest, had moved down into an area where they normally would have had access to water, but which was now severely desiccated.

## DISCUSSION

### Widespread effects of January 2002 storm

Satellite weather images indicate that contrary to early rumors, the January storm occurred throughout central Mexico and broadly impacted the entire overwintering region of the monarchs (see figure 20.1). Heavy rain and snow caused extreme wetting of the clustering butterflies, which reduced their natural cryoprotection. When the weather cleared early in the morning on the 14th, the temperature dropped to about  $-4^{\circ}\text{C}$ , killing a historically unprecedented number of butterflies. The geographic extent and pattern of the storm strongly suggest that all known overwintering colonies in Mexico were severely affected.

### Quantitative estimates of the mortality

Systematic sampling of monarch mortality led to an estimate of 4940 dead monarchs/ $\text{m}^2$ , averaged

across the two colonies sampled (see tables 20.3 and 20.4). Our March visit suggested that mortality in the El Capulin colony was at least as severe as it had been in these two colonies. It therefore seems reasonable to use this average mortality estimate, rounded to 5000 killed/ $\text{m}^2$ , as a conservative estimate for the entire overwintering region.

In December 2001, García-Serrano (2002) estimated that most of the known overwintering colonies comprised a total area of 9.35 ha. Multiplying this by the average mortality per square meter gives an estimated 467.5 million monarchs killed by the storm. Since García-Serrano did not visit all the known overwintering areas (Bojórquez-Tapia et al. 2002), we revise this estimate upward to 500 million monarchs killed across the overwintering region. Averaging the percentage of colony loss across the two colonies sampled (see table 20.4), we estimate an average 75% colony mortality.

The average percentage of monarchs killed in the two colonies was remarkably consistent with the predictions based on the cryobiological experiments of Anderson and Brower (1996). According to their data, 5% of monarchs with water on their bodies freeze to death as the temperature falls to  $-3.0^{\circ}\text{C}$ , 50% die at  $-4.0^{\circ}\text{C}$ , and 80% die at  $-5^{\circ}\text{C}$ . We show that the monarchs were wetted by the storm prior to freezing and that the post-storm (14 January) temperatures in the Zapatero colony dropped to between  $-4^{\circ}$  and  $-5^{\circ}$ . Thus the 50% to 80% mortality predicted by the experimental data was realized by the 75% mortality that we estimated to have occurred in the two colonies.

### Variation in estimates of dead monarchs per square meter

Both natural and anthropogenic factors contribute to the extreme variability in the numbers of monarchs killed per square meter. The main factor was likely heterogeneity of the oyamel tree cover that ranges from large forest gaps to complete canopy closure. Most of our low sample counts were collected from the floor beneath large forest gaps where no butterflies could have been roosting during the storm. The densest samples were from beneath and adjacent to the large healthy oyamels, on which the majority of butterflies roosted. Natural variation occurs in the numbers, size, and quality of available lower branches on the oyamels. There is also variation in the densities of the monarch clusters on the



tree branches and trunks. Other factors might include the position of the colony with respect to the direction from which the storm approached, slope inclination, the degree to which the colony had nestled into an arroyo, the presence of diseased and lightning-struck trees, and the age distribution of the forest.

#### Historical underestimate of the density of monarchs in the overwintering colonies

On first visiting the Zapatero colony in January 1977, Brower conservatively estimated 1000 live monarchs/m<sup>2</sup> or 10 million/ha (Brower 1977; Brower et al. 1977). This value has been used to estimate the size of the fall migratory populations (e.g., García-Serrano et al., this volume). However, the data presented in this chapter indicate that the actual number far exceeds the early estimate (see also Calvert, this volume). We now suggest revision of the numbers of living monarchs in a typical colony based on our estimate of the mean number dead per square meter (5000) and our estimate of average colony mortality (75%). Thus we now suggest that there were 6667 monarchs/m<sup>2</sup> in the pre-storm colony. Rounding to 6500, we estimate 65 million monarchs/ha at Mexican overwintering sites. This revision will require a reevaluation of the published estimates of the sizes of migratory fall populations of eastern North American monarchs (e.g., Taylor 1999; Taylor et al. 2000).

#### The mortality estimation controversy

Lack of understanding of the continued accumulation of dead butterflies over several weeks after the storm and the incomplete data analyses of García-Serrano's collections led to the release of conflicting statements to the Mexican press shortly after the storm. Based on information provided by Roberto Solis, then director of the Monarch Butterfly Biosphere Reserve, Elorriaga (2002a) reported on 25 January that 2 to 5 million, or only 2% to 5% of the monarchs, had been killed in the Rosario and San Andrés colonies. On 8 February, World Wildlife Fund Mexico (in Bezaury and Rojas 2002), having first provided the information to the highest-level officials in SEMARNAP and COANANP (Comisión Nacional de Áreas Naturales Protegidas [National Commission of Natural Protected Areas]), made a formal press release based on our 58 samples

summarizing the much higher mortality. Brower released these data to the *New York Times* for a 12 February report (Yoon 2002). Aware of our findings, Solis revised his estimate upward on 12 February to 35 million, stating that all five of the sanctuaries in Michoacán had been hit, and that about 30% of the known overwintering monarchs had been killed (Elorriaga 2002b). Solis apparently based his revision on the incompletely analyzed data from García-Serrano. Not realizing the difference in the dates on which the samples were collected and the continuing accumulation of dead butterflies on the forest floor, he ascribed our higher estimates to "an error, or bad faith" (Stevenson 2002).

Given the history of confrontation and confusion in the Mexican and international press over the pesticide issue in 2001, we were extremely cautious about the possibility of exaggeration based on our initial mortality estimates. It was exactly for this reason that we performed the systematic transect counts (see table 20.3). We have extensively reviewed our methods and analyses and are confident that these data, although highly variable, do not overestimate the mortality.

#### Post-storm colony movement and effects of human disturbance

Earlier studies in the Sierra Chincua determined that overwintering colonies of monarchs drop in altitude as the headwater streams shrink with the advancing dry season. This colony movement is slow at first but accelerates prior to the butterflies' spring remigration northward (Calvert and Brower 1986) and is almost certainly a desiccation avoidance behavior (Calvert and Lawton 1993).

The present study established a second category of colony response: the storm survivors in the Zapatero, Rosario, Conejos, and Capulin colonies all deserted the area of severe mortality and formed new colonies. Because northern cold fronts regularly cause winter storms in central Mexico and have probably done so back to the Pleistocene, it is likely that monarchs at these high-altitude sites have been under strong selective pressure favoring various adaptive responses. Thus we propose that the post-storm movement is an evolved behavioral response different from the seasonal desiccation movement. One possible adaptive benefit would be to avoid pathogens from the mass decomposition of the millions of carcasses on the forest floor. Another would

be to move to an area of the forest that provides increased canopy shelter. Herein lies a serious conflict because of human encroachment on the forests: if adjacent areas have been thinned, then moving can result in the colony re-forming in a site providing even less protection if another storm impacts the area.

Extensive human disturbance of the forests in and adjacent to the butterfly colonies has been documented elsewhere (Brower et al. 2002) and undoubtedly affects monarch survival in all the known overwintering massifs. For example, during the dry El Niño spring of 1998, agricultural carelessness caused a forest fire that spread up the Sierra Chincua ridge into the southwestern rim of Arroyo Zapatero (Brower and Missrie 1998). This, plus a wide boundary cut and prior forest thinning on the colony's border area, increased the exposure of the Zapatero colony to the January 2002 storm. The aforementioned degradation of the forest and hydrology on Cerro Pelón and our observation that the surviving remnant of the Rosario colony moved about 200 m laterally into a sparsely forested area further illustrate effects of human-induced disturbance. The important forest on the edge of this principal ecotourism colony has been subjected to incremental illegal tree removal over the past several years (L. Brower, pers. observ.). Finally, the survivors in the Conejos colony moved upslope into an area that had been heavily thinned by selective cutting in March 1989 (L. Brower, pers. observ.).

More generally, the effects of this severe weather event in January 2002 support predictions of the relationship between weather events, forest conditions, and overwintering monarch mortality. The larger picture is that a vegetation analysis documented that the forest in the area surrounding both colonies was seriously degraded between 1986 and 1999 (Brower et al. 2002). These facts are a resounding wake-up call for more effective protection of the Monarch Butterfly Biosphere Reserve.

#### Call for a stronger Mexico research program

Our study demonstrates the need to establish a more effective ongoing research program that monitors the overwintering colonies and collects basic data needed to responsibly manage the Monarch Butterfly Biosphere Reserve. As a start, electronic weather-monitoring stations should be

installed in the overwintering colonies, and the methodology for monitoring and estimating mortality within the colonies needs to be standardized. Employment of trained local research biologists, in strong collaboration with the international scientific community, would also help, as would renovation of the Sierra Chincua research station.

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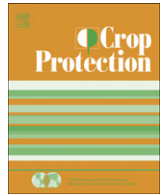
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## Short Communication

Reduction in common milkweed (*Asclepias syriaca*) occurrence in Iowa cropland from 1999 to 2009

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## ABSTRACT

The role of common milkweed in the lifecycle of the monarch butterfly has increased interest in the presence of this weed in the north central United States. An initial survey conducted in 1999 found that low densities of common milkweed occurred in approximately 50% of Iowa corn and soybean fields. In 2009, common milkweed was present in only 8% of surveyed fields, and the area within infested fields occupied by common milkweed was reduced by approximately 90% compared to 1999. The widespread adoption of glyphosate resistant corn and soybean cultivars and the reliance on post-emergence applications of glyphosate for weed control in crop fields likely has contributed to the decline in common milkweed in agricultural fields.

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## 1. Introduction

Common milkweed is a perennial native to northeastern and north central United States (US) and adjacent areas of Canada (Bhowmik and Bandeen, 1976). The plant is adapted to a variety of habitats, and is found in cropland, pastures, prairies and roadsides. Members of the Asclepiadaceae family are the sole food source of the monarch butterfly (*Danaus plexippus*) larvae, whereas adult monarchs feed on a variety of flowers (Brower, 1969). While numerous *Asclepias* species are found in Iowa and surrounding area, common milkweed is the most prevalent and considered the preferred source of monarch larvae in the region.

Common milkweed is frequently found in agricultural fields (Cramer and Burnside, 1982; Hartzler and Buhler, 2000). Although common, it rarely reaches population densities that impact crop yield and typically does not drive weed management decisions. However, since approximately 75% of Iowa land is involved in agricultural production, the majority of common milkweed in Iowa is found in crop fields. Interest in the distribution of common milkweed has increased in recent years due to its relationship with monarch butterflies, the finding that 50% of wintering monarchs originated from the north central United States (Wassenaar and Hobson, 1998), and concerns regarding potential impacts of transgenic crops on the fate of the butterfly.

Shortly after the introduction of transgenic BT (*Bacillus thuringiensis*) corn, it was reported that pollen from some of these transgenic hybrids contained sufficient BT toxin to pose a threat to monarch larvae (Hansen and Obrycki, 2000). Monarchs were found to utilize common milkweed in corn fields, and larvae densities on common milkweed were as high or higher in agricultural fields as in non-agricultural habitats (Oberhauser et al., 2001). Later research found that most BT hybrids expressed the toxin in pollen at concentrations unlikely to have severe fitness penalties to larvae feeding on milkweed within corn fields (Hellmich et al., 2001).

Since the majority of common milkweed in Iowa, and likely the north central US, is found in crop fields, and these plants are an important resource for monarchs, changes in weed management systems that increase common milkweed control could have an indirect effect on the monarch (Oberhauser et al., 2001). Soybean cultivars genetically modified for resistance to glyphosate (GR) were introduced in 1996 and the majority of US soybean acres were GR by 1999 (Dill et al., 2008). By 2006, 96% of US soybean possessed the GR trait. Although adoption of GR corn was much slower than that of soybean, approximately 35% of the US corn acres were planted with GR corn hybrids in 2006. Seventy-five percent of Iowa farmers reported planting continuous GR crops in a 2006 survey (Shaw et al., 2009). Due to the systemic characteristics of glyphosate, repeated use of glyphosate in GR crops could reduce infestations of perennial weeds such as common milkweed (Duke and Powles, 2008). To access the impact of changing weed management practices on common milkweed prevalence, crop fields and

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**Table 1**

Occurrence of common milkweed in Iowa roadsides and crop fields (corn and soybean).

Land use	Number of sites surveyed		Sites infested (%)		Area infested $\pm$ SD ( $\text{m}^2 \text{ha}^{-1}$ )	
	1999 <sup>a</sup>	2009	1999 <sup>a</sup>	2009	1999 <sup>a</sup>	2009
Roadside	407	216	71	82	102 $\pm$ 238	96 $\pm$ 242
Crop field	332	216	51	8	52 $\pm$ 51	5 $\pm$ 5

<sup>a</sup> 1999 data adapted from Hartzler and Buhler (2000).

adjacent areas in Iowa were surveyed for the presence and abundance of common milkweed in the summer of 2009.

## 2. Materials and methods

The presence of common milkweed in corn and soybean fields, and the adjacent roadside, was determined by a plant census conducted during June and July of 2009. The timing of the survey was designed to assess common milkweed infestations after the crop and weed had emerged but prior to post-emergence herbicide applications. Since common milkweed emerging from vegetative rootstocks is not significantly affected by pre-emergence herbicides used in corn and soybean, this allowed an accurate measurement of infestations within crop fields.

The sampling protocol was similar to a 1999 survey (Hartzler and Buhler, 2000), although a different method was used to generate a random sample. Iowa is divided into nine Crop Reporting Districts via two vertical and two horizontal transects along county lines. Counties within Iowa are divided into townships of approximately 94km<sup>2</sup>. Four townships were randomly selected within each Crop Reporting District, and then plat maps were used to locate six sampling areas within each selected township. A total of 216 sites were sampled in 2009. Sampling areas were identified prior to going to the field by arbitrarily specifying a distance from a landmark, usually a road intersection. In situations where the designated area was not planted to corn or soybean, the first field encountered beyond the designated area was surveyed. This method eliminated sampling bias since the sampling areas were selected without prior knowledge of the terrain or vegetation.

A 50 m section of the roadside was surveyed for common milkweed, the width of the roadside at most sites was approximately 10 m and the dominant roadside vegetation was smooth brome (*Bromus inermis* Leyss.). A 50 m by 100 m area of the adjoining crop field was then surveyed for the presence of common milkweed. Data collected included vegetation type, crop growth stage, number of distinct common milkweed patches, and size of individual common milkweed patches. Common milkweed stems within 1 m of each other were considered to be part of a patch, and patch size was estimated as the area encompassed by contiguous stems. Solitary stems were assigned a patch size of 1 m<sup>2</sup>.

Analysis of variance was used to compare common milkweed infestations among different habitats. Data presented are percentage of sites infested with common milkweed and cumulative area infested with common milkweed. Sites not infested with common milkweed were not included in the data set when calculating cumulative area infested.

## 3. Results and discussion

Common milkweed occurrence did not vary across the state of Iowa, thus data from the nine Crop Reporting Districts were combined. In addition, data from corn and soybean field were pooled due to lack of a significant difference in infestations between the two crops.

Eighty-two percent of roadsides surveyed had common milkweed present in 2009, compared to 71% of roadsides in 1999

(Table 1). Although the Iowa land area maintained in roadsides is relatively small (approximately 330,000 ha), it is uniformly distributed across the landscape and may be important as an oviposition site for monarchs. A survey in Nebraska in the early 1980s reported 51% of roadsides infested with common milkweed (Cramer and Burnside, 1982). Herbicide use on Iowa roadsides has declined since the establishment of an integrated roadside vegetation management program in 1988 (Flynn, 1994), which likely contributes to the high frequency of occurrence of common milkweed in this habitat. Roadsides are typically only sprayed with herbicides when weeds designated as noxious by the state are present.

The percentage of crop fields infested with, and the amount of common milkweed present in infested fields declined in the time between the two surveys (Table 1). In 1999 common milkweed was found in 51% of the crop fields, whereas in 2009 only 8% of the fields were infested with the weed. In several of the infested fields, common milkweed was only found within a few m of large common milkweed patches present in the adjacent roadside (data not presented). The area occupied by common milkweed patches in infested fields declined by approximately 90% from 1999 to 2009.

A large reduction in common milkweed in Iowa corn and soybean fields occurred between 1999 and 2009. Although several factors could have contributed to this decline, the widespread adoption of GR crops and concomitant applications of glyphosate are likely primary contributing factors. A survey of cropping systems employed by Iowa farmers found 75% of crop rotations used in the state consisted of continuous GR crops (Shaw et al., 2009).

Iowa's landscape is dominated by cropland, with approximately 75% of the landmass dedicated to agricultural production. It was estimated that Iowa corn and soybean fields produced 78 times more monarchs than non-agricultural habitats (Oberhauser et al., 2001). Thus, the decline in common milkweed found in corn and soybean fields could affect monarch reproduction within Iowa and surrounding states with similar land use patterns. The land areas most frequently infested with common milkweed were roadsides and areas enrolled in the Conservation Reserve Program (Hartzler and Buhler, 2000), but approximately 1 million hectares are dedicated to these land uses compared to 9.1 million hectare planted to corn and soybean. Estimates of monarch wintering populations in Mexico over the time frame of the common milkweed surveys do not indicate a decline in butterflies that parallels that of common milkweed ([www.monarchwatch.org](http://www.monarchwatch.org)). Rather, fluctuations in monarchs were reported to correlate with climatic events that influenced survival and reproduction of the monarch. Thus, the long-term impact of declining common milkweed populations in Iowa on monarchs is difficult to access.

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## FORUM

# Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk?

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**Abstract.** 1. During the 2009–2010 overwintering season and following a 15-year downward trend, the total area in Mexico occupied by the eastern North American population of overwintering monarch butterflies reached an all-time low. Despite an increase, it remained low in 2010–2011.

2. Although the data set is small, the decline in abundance is statistically significant using both linear and exponential regression models.

3. Three factors appear to have contributed to reduce monarch abundance: degradation of the forest in the overwintering areas; the loss of breeding habitat in the United States due to the expansion of GM herbicide-resistant crops, with consequent loss of milkweed host plants, as well as continued land development; and severe weather.

4. This decline calls into question the long-term survival of the monarchs' migratory phenomenon.

**Resumen.** 1. Durante la temporada invernal 2009–2010, y siguiendo una tendencia a la baja de 15 años, la superficie total ocupada por mariposas monarca en México, provenientes del este América del Norte, llegó a su punto más bajo. A pesar de su incremento, dicha superficie siguió siendo baja en 2010–2011.

2. Aunque que el conjunto de datos disponibles es aún pequeño, esta disminución de la abundancia de mariposas es estadísticamente significativa, tanto si se usan modelos de regresión lineales como exponenciales.

3. Hay tres factores que parecen haber contribuido con esta tendencia de reducción del número de mariposas: la degradación de bosque en las áreas de invernación en México; la pérdida de hábitat de reproducción en los Estados Unidos, debido a la expansión de cultivos genéticamente modificados resistentes a herbicidas, con la consiguiente pérdida de las plantas hospedadoras de algodoncillo, y por continuos cambios en el uso del suelo no favorables para ellas; y, las recientes condiciones climáticas severas.

4. Esta disminución hace que nos cuestionemos sobre la posibilidad de supervivencia a largo plazo del fenómeno migratorio de las mariposas monarca.

**Key words.** Conservation, endangered biological phenomenon, habitat, Lepidoptera, migration, monitoring.

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### The decline in monarch abundance

Wilcove (2008) has warned of the potential collapse of numerous animal migrations, including the unique migration and overwintering biology of the eastern North American population of the monarch butterfly, *Danaus plexippus* L. (Lepidoptera: Danaidae). During the 2009–2010 season and following a 15-year downward trend, the total area of overwintering colonies reached an all-time low (Rendón-Salinas *et al.*, 2010; Fig. 1). Yearly monarch abundance is assessed by measuring the combined area occupied by all known overwintering colonies in Mexico, and these data have been published online by World Wildlife Fund-Mexico since the 1994–1995 overwintering season, with data to 2001 also available in Garcia-Serrano *et al.* (2004). The average area occupied by the butterflies over the past 17 years is 7.24 ha, with a maximum of 20.97 ha during the 1996–1997 season and a minimum of 1.92 ha during the 2009–2010 season, and recovery to only 4.02 ha during the 2010–2011 season (Rendón-Salinas *et al.*, 2011). The 1996–1997 overwintering season was monitored by Garcia-Serrano and Mora-Alvarez (1999) and also by a separate federal team of investigators (PROFEPA) (reference in Bojorquez *et al.*, 2003), and we are confident that it was the largest recorded over the 17 years for which we have an adequate database. All of the past 7 years have been below the 17-year average. We have analysed these data and found that the decline is statistically significant.

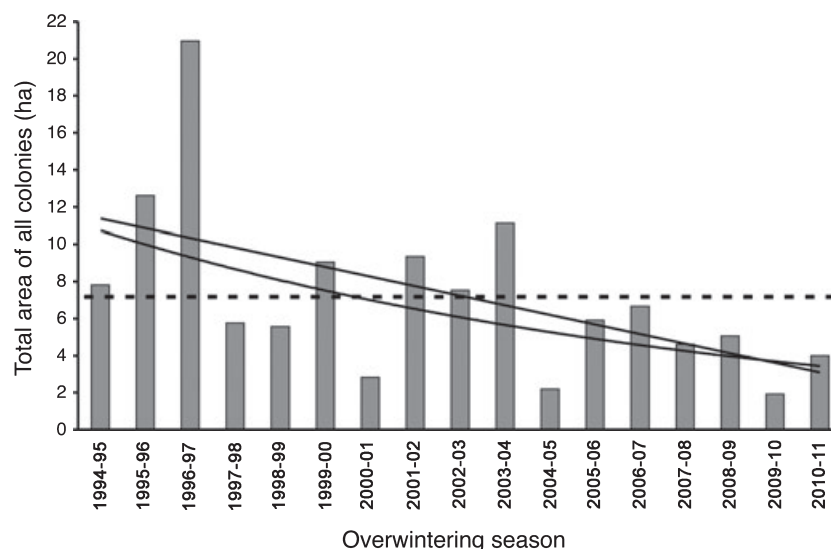
To assess the time dependency of the measurements of colony area, we considered two regression models using the 17 years' data from Rendón-Salinas *et al.* (2010, 2011), with 1994 as year 1: a linear model, because it provides the simplest relationship between the time and area variables, and an exponential model, because it is the model frequently used to analyse population growth. We first examined the data for independence of successive years' measurements and found no evidence of autocorrela-

tion (Durbin–Watson test, 4-d = 1.538, with critical dU = 1.371;  $P > 0.05$ ). Both linear and exponential regressions showed a significant decline in total colony area (Fig. 1; analysed with SPSS 2010): the linear model  $y = 11.89 - 0.52x$  was significant at  $P = 0.018$ , with  $F_{1,15} = 6.989$ , and the exponential model  $y = 11.52e^{(-0.071x)}$  was significant at  $P = 0.015$ , with  $F_{1,15} = 7.601$ . The  $r^2$  values were 0.318 and 0.336, respectively. We also ran polynomial regression models, but they did not increase  $r^2$ .

Reliable information on colony sizes and locations is available since the 1994–1995 overwintering season; earlier information was gathered on increasing numbers of colonies as they were discovered by diverse groups of investigators with variable expertise. Even though the data span only 17 years, the decline is statistically significant. The regressions remain significant when either extreme measurement (high in 1996–1997 or low in 2009–2010) is removed (linear model,  $P = 0.032$  or 0.042; exponential model,  $P = 0.040$  or 0.049). We believe that all the measurements we have analysed are reasonably reliable. Continued monitoring will, of course, strengthen conclusions about trends in monarch abundance.

### Factors leading to declining abundance

Three factors are implicated in the downward trend in the monarch's abundance: (i) the loss of and reduction in quality of critical overwintering habitat in Mexico through extensive illegal logging; (ii) the widespread reduction of breeding habitat in the United States due to continuing land development and the killing of the monarch's principal larval foodplant, the common milkweed *Asclepias syriaca* L. (Asclepiadaceae), because of increased use of glyphosate herbicide to kill weeds growing in genetically engineered, herbicide-resistant crops; and (iii)



**Fig. 1.** The total annual area occupied by overwintering monarch butterflies from 1994 through 2011 has declined significantly, with the all-time smallest area reported during the 2009–10 overwintering season. The dashed line shows the 17-year average (7.24 ha). Both linear (upper) and exponential (lower) regression lines are included (see text for regressions).

periodic extreme weather conditions, such as those that occurred most recently in 2009, that decrease both the spring breeding in Texas and the subsequent spring and summer breeding generations in the eastern USA and southern Canada.

### Forest degradation

On the 12 known massifs that host the butterfly colonies in Mexico (Slayback *et al.*, 2007), illegal logging has eliminated overwintering habitats on several and severely degraded them on others. For example, between 1971 and 1999, 44% of the high quality over-wintering forest was degraded within the area that became protected as the Monarch Butterfly Special Biosphere Reserve by presidential decree in 1986 (Brower *et al.*, 2002). Then, between 2001 and 2009, after the new 2000 presidential decree enlarged the Reserve core zone to 13 552 ha, 1349 ha (10%) were severely degraded or clear cut (Anonymous, 2009). Colony areas that have been entirely lost include several on the north face of Cerro Pelon (Ramirez *et al.*, 2008; L.P. Brower & D. Slayback, unpubl. aerial reconnaissance and satellite imagery) and at least three areas in the Lomas de Aparacio area on the southern portion of the Sierra Campanario (Brower *et al.*, 2008). Colony areas that have been logged to the point at which few monarchs still aggregate include the west face of Cerro Pelon and the south face of Cerro Altamirano. Even the two principal ecotourism colony areas, Rosario and the Sierra Chincua, have been degraded by incremental logging over the past two decades (L.P. Brower, in prep.).

### Loss of breeding habitat in the United States

Seiber *et al.* (1986) and Malcolm *et al.* (1993) determined through thin layer chromatography that 85 and 92%, respectively, of 394 and 382 overwintering monarch butterflies in Mexico had fed as larvae on the Common Milkweed, *Asclepias syriaca*. The importance of *A. syriaca* reflects history of the landscape. A rich pre-colonial milkweed flora was widely distributed, with 29 species of *Asclepias*, most of them grassland species (Woodson, 1954; Hartman, 1986) native to the late summer breeding range of the monarch (Malcolm *et al.*, 1989, 1993; Wassenaar & Hobson, 1998). However, ploughing of the prairies and deforestation led to an increase in the distribution and abundance of *A. syriaca* (Brower, 1995), which Woodson referred to as the pre-eminent weedy North American milkweed. Now with an increasingly patchy distribution, this species is the dominant milkweed in the monarch's eastern North American breeding range.

A survey in 1999 of habitats containing this milkweed species showed that the number of monarchs produced per ha in maize (corn) and soya (soybean) fields was as high or higher than that of other habitats (Oberhauser *et al.*, 2001). Genetically modified glyphosate resistant (GR) soya and maize (e.g. Monsanto's Roundup Ready crops) were rapidly adopted by growers after 1999, resulting in a significant reduction of *A. syriaca* and the loss of monarch breeding habitats in these croplands. Much of the combined acreage of soya and maize (60–70 million ha per

year) is used in rotation, and this rotation in combination with the high adoption rate of GR soya (> 70% by 2002, presently 92%) and maize (presently 23%) (U.S.D.A., 2010a) has all but eliminated *A. syriaca* from 40 million ha of these croplands (Taylor, 2008). Both Hartzler (2010) and J.M. Pleasants (in prep.) have documented the drastic reduction of *A. syriaca* growing in glyphosate-treated fields in Iowa; Hartzler recorded a 90% loss from 1999 to 2009, and Pleasants measured a 79% loss from 2000 to 2009. We conclude that, because of the extensive use of glyphosate herbicide on crops that are genetically modified to resist the herbicide, milkweeds will disappear almost completely from croplands. Furthermore, Zalucki and Lammers (2010) have estimated with models that the large-scale elimination of milkweeds in agricultural and surrounding landscapes has the effect of increasing the search time for host plants by monarch females with the result that realised fecundity is reduced.

In addition, milkweed habitat has been lost due to increasing demand for biofuels. Conservation Reserve Program (CRP) area has been decreased by 2.3 million ha since 2006 (U.S.D.A., 2010b) and as yet undetermined but large areas of grassland and rangeland have been converted to biofuel crops, especially maize (Stubbs, 2007). Over this same interval, maize and soya planting increased by more than 5 million ha (U.S.D.A., 2010a, and previous year reports from the USDA National Agricultural Statistics Service). Coupled with the habitat lost to development, which has been calculated as nearly 1 million ha each year from 1992 to 2007 (U.S.D.A., 2003, 2009), these losses add to at least 56 million ha (roughly 220 000 square miles). This is more than one-fifth of the estimated eastern North American summer breeding range of the monarch (Brower, 1999). The cost to the monarch population of habitat loss due to GR crops, increased planting of maize and soya, in addition to development is surely significant.

### Extreme weather

Severe cold threatens the survivorship of overwintering monarchs, and spring and summer weather that is too cold or too hot lowers breeding season survivorship and fecundity and alters larval growth rates. In the spring of 2009, first-generation monarchs in Texas were negatively affected in March by above normal temperatures. Subsequent low temperatures in the corn (maize) belt, the third lowest in 42 years, limited growth of the summer generations. These climatic factors severely reduced the numbers of butterflies in the fall migration to Mexico (Taylor, 2009).

Then, during the 2009–2010 overwintering season, the butterflies were subjected to a record-breaking amount of precipitation during the dry season (Brower *et al.*, 2010). From 31 Oct 2009 through 31 Mar 2010, 577 mm of precipitation fell, compared to 40 mm and 20 mm over the same time span for the previous two seasons, as recorded by the electronic weather station (Model 232; WeatherHawk, Logan, UT, USA) we established on the Sierra Chincua at the El Llano las Papas Field Station (100°16'5"W, 19°39'42"N). This station is at the same elevation (3160 m) and 2–4 km from a principal overwintering area



(Brower *et al.*, 2009). From 31 January to 4 February 2010, the WeatherHawk recorded 360 mm of rain. Associated heavy winds blew down hundreds of oyamel fir trees in the core zone of the Reserve (pers. obs., Mar 2010). A low temperature of  $-3.2^{\circ}\text{C}$  occurred as the skies cleared immediately after the storm; the second morning, after the butterflies had dried, the temperature dropped to  $-6^{\circ}\text{C}$ .

The 5-day storm caused major flooding, landslides, structural damage, and loss of human life (Elorriaga, 2010). Local observers (e.g., Rodriguez, 2010) reported that high winds associated with this and several less severe storms scattered the butterflies from their bough and trunk clusters. According to Anderson and Brower (1996), the low temperature recorded immediately after the storm could have killed 5–10% of the wetted butterflies. Had the drop to  $-6.0^{\circ}\text{C}$  occurred while the butterflies were still wet, rather than on the second morning when they were dry, more than 90% mortality could have occurred. Interviews of scientists, tour leaders, and tourists who visited the overwintering monarch colonies subsequent to the storm led Taylor (2010) to conclude that 50% of the monarchs died. Thus, this storm, combined with the lowest number of overwintering monarchs yet recorded, could have resulted in such a reduction in the number of remigrating spring butterflies that recovery of the eastern North American population would have required several breeding seasons. Nevertheless, the butterflies increased to 4.02 ha in 2010–2011, although this value is still well below the 17-year average of 7.24 ha. The frequency of severe precipitation events is likely to increase with climate change (Oberhauser & Peterson, 2003; IPCC, 2007).

## Outlook

The unique migratory phenomenon of the monarch butterfly has been designated as an endangered biological phenomenon (Brower & Malcolm, 1991). Concerns about breeding habitat and overwintering forest habitat loss were central issues in a Commission for Environmental Cooperation conference held in Morelia during December 2007 that led to the North American Monarch Butterfly Conservation Plan (Oberhauser *et al.*, 2008). Increasing international interest in the North American monarch phenomenon also led to the designation on 8 July 2008 of the Monarch Butterfly Biosphere Reserve as a World Heritage Site (Anonymous, 2008). In this paper, we have presented an analysis of the long-term trend in monarch abundance, a decline that exists despite some fluctuation year-to-year. The combination of lowered numbers of fall migrants, the illegal logging in the overwintering region, the severe losses of breeding habitat due both to GM crops and development, and the near miss of catastrophic mortality by the 2010 storm suggest that better stewardship is needed to assure the future of the monarch migratory phenomenon.

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# Milkweed loss in agricultural fields because of herbicide use: effect on the monarch butterfly population

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**Abstract.** 1. The size of the Mexican overwintering population of monarch butterflies has decreased over the last decade. Approximately half of these butterflies come from the U.S. Midwest where larvae feed on common milkweed. There has been a large decline in milkweed in agricultural fields in the Midwest over the last decade. This loss is coincident with the increased use of glyphosate herbicide in conjunction with increased planting of genetically modified (GM) glyphosate-tolerant corn (maize) and soybeans (soya).

2. We investigate whether the decline in the size of the overwintering population can be attributed to a decline in monarch production owing to a loss of milkweeds in agricultural fields in the Midwest. We estimate Midwest annual monarch production using data on the number of monarch eggs per milkweed plant for milkweeds in different habitats, the density of milkweeds in different habitats, and the area occupied by those habitats on the landscape.

3. We estimate that there has been a 58% decline in milkweeds on the Midwest landscape and an 81% decline in monarch production in the Midwest from 1999 to 2010. Monarch production in the Midwest each year was positively correlated with the size of the subsequent overwintering population in Mexico. Taken together, these results strongly suggest that a loss of agricultural milkweeds is a major contributor to the decline in the monarch population.

4. The smaller monarch population size that has become the norm will make the species more vulnerable to other conservation threats.

**Key words.** Glyphosate, GMO, milkweed, monarch butterfly

## Introduction

Monarch butterflies (*Danaus plexippus* L. Lepidoptera: Danainae) in the Eastern North American migratory population undergo a multi-generation annual cycle that includes wintering in central Mexico. In the spring, adults that have overwintered migrate north and reproduce in Texas and states to the north and east. Their offspring move farther north into much of the eastern half of the United States and southern Canada, and two to three more generations are produced (Cockrell *et al.*, 1993; Malcolm *et al.*, 1993; Prysby & Oberhauser, 2004). Most adults that emerge after mid-August are in a state of reproductive diapause (Herman, 1985; Goehring & Oberhauser, 2002) and

migrate from the summer breeding range to their wintering grounds, where they remain until spring (Solensky, 2004).

Annual counts of the size of the overwintering population in Mexico indicate that the monarch population has been declining over the last decade and a half (Rendón-Salinas *et al.*, 2011; Brower *et al.*, 2011b). One possible explanation for this decline is that monarch production has been decreasing as a result of a reduction in the availability of the larval host plant. Monarch larvae feed primarily on milkweeds (genus *Asclepias*- Family *Apocynaceae*, subfamily *Asclepiodeae*). On the basis of milkweed cardenolide fingerprints, it has been estimated that 92% of the monarchs wintering in Mexico had fed as larvae on the common milkweed, *Asclepias syriaca* (Malcolm *et al.*, 1993). Studies in Iowa found a large reduction in *A. syriaca* in corn (maize, *Zea mays*) and soybean (soya, *Glycine max*) fields from 1999 to 2009 (Hartzler & Buhler, 2000; Hartzler, 2010). It is likely that a similar reduction has occurred throughout the region where corn and soybeans are predominantly grown. Eighty per cent of both

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corn and soybeans are grown in the Midwest (USDA, National Agricultural Statistics Service, 2011c), which is composed of the states of North and South Dakota, Nebraska, Kansas, Missouri, Iowa, Minnesota, Wisconsin, Illinois, Indiana, Michigan, and Ohio. A study in 2000 (Oberhauser *et al.*, 2001) found that monarchs heavily used milkweeds in corn and soybean fields. On the basis of stable isotope analysis, Wassenaar and Hobson (1998) estimated that half of the monarchs overwintering in Mexico in 1997 came from the Midwest. Thus, the Midwestern United States is at the epicentre of a reduction in milkweeds in agricultural fields and is also an area that has in recent history contributed a large component of the monarch population. In this study, we estimate the magnitude of this milkweed loss and its consequences for monarch production.

Milkweed in agricultural fields has long been a concern for farmers as its presence reduces yield (Bhowmik, 1994). In the 1970s and 1980s, milkweed infestation in agricultural fields was viewed to be on the increase with 10.5 million ha infested in the north-central states (Martin & Burnside, 1980). Herbicides have been increasingly used to control weeds in row crops. Many of these herbicides produce only moderate control of milkweed, but glyphosate, often referred to as Roundup™ (Monsanto, St. Louis, MO, USA), is more effective (Bhowmik, 1994; Pline *et al.*, 2000). However, it also has a detrimental effect on crop plants, so until the development of genetically modified (GM) glyphosate-tolerant (Roundup Ready™, Monsanto) crop plants, herbicides other than glyphosate were used to control weeds. Glyphosate-tolerant soybeans were introduced in 1996 and had reached a 94% adoption level by 2011, and glyphosate-tolerant corn was introduced in 1998 and had reached a 72% adoption level by 2011 (USDA, Economic Research Service, 2011). Glyphosate use in soybeans went from 1.4 million kg in 1994 to 41.7 million kg in 2006 (the last year for which data are available and when adoption of glyphosate-tolerant soybeans was 89%) and glyphosate use in corn went from 1.8 million kg in 2000 to 28.5 million kg in 2010 when the adoption level was 70% (USDA, National Agricultural Statistics Service, 2011a,b).

The time period (1999–2009) over which the Iowa studies found a large reduction in *A. syriaca* in corn and soybean fields (Hartzler & Buhler, 2000; Hartzler, 2010) is coincident with the period when use of glyphosate herbicide increased in conjunction with the increased adoption of glyphosate-tolerant corn and soybeans. It is very probable that a similar milkweed reduction has occurred throughout the Midwest because adoption levels of herbicide-tolerant crops are similar throughout this region (USDA, Economic Research Service, 2011). How much milkweed loss does this represent on a landscape scale? To address this question, we need information on the density of milkweeds in different habitats and the landscape area covered by those habitats. Common milkweed tends to be found in habitats with a moderate degree of disturbance, including roadsides, pastures, old fields, prairies and agricultural fields (Bhowmik, 1994). Multiple data sets provide information on the density of milkweeds in different habitats over the last decade. The studies by Hartzler and Buhler (2000) and Hartzler (2010) surveyed a number of milkweed habitats in Iowa, including agricultural fields. Additionally, a number of Midwest volunteers in the Monarch Larva Monitoring Project (2011), hereafter referred to as MLMP,

measured milkweed density in their non-agricultural observation patches over several consecutive years. Milkweed density data can be combined with published statewide land-use data to estimate the number of milkweeds in different habitats. Some of the data sets we use come from Iowa because for some parameters only Iowa data are available. However, we use data from the Midwest as a whole whenever possible and make the case that the resulting estimates of monarch production are representative of the Midwest.

What is the significance of the loss of milkweeds in agricultural fields for monarchs? To address this issue, we need to estimate annual monarch production in the Midwest over the last decade to determine whether there has been a significant downward trend. Obtaining data to estimate production is difficult, despite the fact that the monarch butterfly is such a well-studied species. One approach would be to use the number of migrants that come out of the Midwest at the end of the summer as a measure of production. A monarch tagging programme begun 20 years ago (Monarch Watch, 2011) has been tracking migrating butterflies. The number of monarchs tagged shows a decline from 2004 to 2010 (Brower *et al.*, 2011a). However, it is difficult to obtain accurate measures of production from this tagging programme because of the variability among the years in the number of person-hours involved in capture and tagging, the fall conditions when tagging occurred and the locations where tagging occurred. Alternatively, one could use counts of the number of migrating monarchs passing particular locations where they tend to be funnelled because of passage over water or geography. Such counts have been made for over a decade in upper Michigan and New Jersey (Davis, 2011) but these sites do not monitor monarchs from the Midwest.

Rather than trying to count adults, another approach to estimating Midwest monarch production is to focus on the number of eggs and larvae found on milkweed plants. This requires monitoring many patches of milkweed in different habitats, including agricultural fields. Production can then be estimated from the average number of monarchs per plant in each habitat and the number of milkweeds in each habitat on the landscape. We have combined several existing data sets that provide this information. The MLMP (2011), which has been operational for over a decade, provides data on egg and larva density on milkweeds. MLMP volunteers are located throughout the monarch breeding range and monitor sites of their choosing weekly over the summer months, reporting the number of plants (stems) monitored and the number of eggs and larvae observed. They learn the procedures of the project through workshops, by reading directions on the project website (MLMP, 2011) and via communication with the project managers (Prysky & Oberhauser, 2004). The sites they monitor, however, are not in agricultural fields. But one of us (Pleasants) has monitored eggs and larvae on milkweeds in both agricultural fields and non-agricultural habitats for several years in central Iowa and a study with larger spatial scale quantified monarch density in both agricultural and non-agricultural habitats in 2000 (Oberhauser *et al.*, 2001). We will make the case that the relative use of milkweeds in agricultural and non-agricultural habitats observed over those years can be extrapolated to provide



data on monarch use of agricultural milkweeds in years where only MLMP data exist. There is a question of what aspect of production to use to estimate monarch population changes. The latest stage for which we have density data, and thus which is closest to *actual* production of adult monarchs, is the fifth instar (L5, the last larval instar). However, there are many factors that can affect survivorship from egg to L5 that have nothing to do with milkweed availability, such as predation and weather. Our goal was to examine the effect of milkweed resource limitation on monarch production. Consequently, we chose to focus on eggs per plant that represents *potential* production.

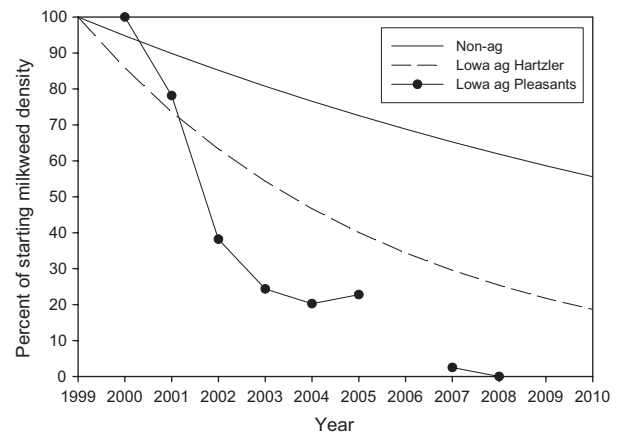
## Methods

### *Data sources for milkweed density*

Habitats in which milkweeds are found include primarily roadsides, corn fields, soybean fields, pastures, old fields, and land set aside from farming and enrolled in the Conservation Reserve Program (CRP). CRP land is typically planted to a variety of cover plants including grasses and forbs. To estimate milkweed densities in these habitats, we used data from several sources: Iowa censuses carried out in 1999 and 2009 (Hartzler & Buhler, 2000; Hartzler, 2010), and data from some MLMP volunteers who measured milkweed density at their sites in several Midwest states. To calculate monarch production for each year, it is necessary to know how milkweed densities have changed over the last decade in non-agricultural and agricultural habitats.

*Non-agricultural habitats.* For roadsides, there was little observed change in milkweed density in Iowa between 1999 and 2009 (Hartzler, 2010) so we have assumed that milkweed density did not change in that habitat over the entire period of the analysis. Hartzler (2010) measured milkweed densities for CRP land and pastures in 1999 but not in 2009 so any change that may have occurred could not be determined from the Iowa data. However, a subset of MLMP volunteers ( $n = 16$ ) measured milkweed density at their sites (which included natural areas, CRP land, pastures and old fields) for at least 4 years over this period (97 total observations). Measurements by individual MLMP volunteers did not cover the entire period but there were sufficiently long and overlapping sequences to provide a complete picture. Volunteers either measured the area of their site and did a complete count of milkweed stems, or used a modified belt transect to sample milkweed density in  $100 \times 1 \text{ m}$  plots. We have used those data to estimate the change in milkweed density in CRP land and pasture land over the last decade.

For the data from the MLMP volunteers, we used log of milkweed density as the variate and used an SAS mixed model and restricted maximum likelihood estimation with fixed effects being ‘habitat’, ‘year’ and ‘habitat by year’. We did not find a ‘habitat by year’ effect so we reran the analysis with this removed. There was a significant ‘year’ effect ( $F_{1,85} = 9.35$ ,



**Fig. 1.** Decline of milkweeds in agricultural and non-agricultural habitats. The line depicting the decline in non-agricultural habitats is based on a regression using data from MLMP volunteers. The line depicting the decline in agricultural habitats is based on an exponential decay function connecting the 1999 and 2009 values from the Iowa surveys (see Methods). Also shown is the proportional change in the number of milkweed stems in all monitored plots in seven agricultural fields in Iowa starting with 998 stems in 2000. The increase in milkweed stems observed in the agricultural sites in 2005 was attributed to the influence of fields where corn was planted 2 years in a row. Some agricultural fields received glyphosate herbicide treatment and others non-glyphosate treatment. No observations were made in 2006.

$P = 0.003$ ). The slope of the regression (on a log scale) was  $-0.0536$ , which corresponds to a decline in density of 5.2% per year. We found no ‘habitat’ effect so we applied the same rate of decline to both CRP land and pastures (Fig. 1).

*Agricultural habitats.* We have values for milkweed density in Iowa agricultural fields for 1999 and 2009 (Hartzler & Buhler, 2000; Hartzler, 2010). To calculate milkweed density in fields for the intervening years, we have to make an assumption about the shape of the decline. Pleasants observed the change in the number of milkweeds in plots in seven agricultural fields in Iowa from 2000 to 2008 (Fig. 1). The observed decline is best described by an exponential decay function. Such a function is also consistent with more acres of glyphosate-tolerant corn and soybeans being planted each year over the last decade (USDA, Economic Research Service, 2011). We have therefore assumed that milkweed density in fields decreased as an exponential decay function from its 1999 value to its 2009 value (see Table 1). This corresponds to a 14.2% decline per year (Fig. 1). Other decline functions, ranging from a linear decline to a more precipitous exponential decay, had no significant effect on the overall results.

### *Data sources for land use*

We obtained data on the acres occupied by roadsides and pastures on the Iowa landscape in 2002 from Lubowski *et al.* (2006) and, because no more recent data exist, we have assumed the

**Table 1.** Estimates of the amount of milkweed in non-agricultural habitats, agricultural fields and total milkweeds in Iowa from 1999 to 2010.

Year	Milkweeds in non-agricultural habitats				Total non-ag mlkws¶
	CRP hectares*	CRP mlkws†	Pasture mlkws‡	Roadside mlkws§	
1999	601	127.4	19.8	38.2	185.4
2000	647	130.1	18.8	38.2	187.1
2001	729	139.0	17.8	38.2	195.0
2002	755	136.4	16.9	38.2	191.5
2003	762	130.5	16.0	38.2	184.7
2004	767	124.5	15.2	38.2	177.9
2005	776	119.5	14.4	38.2	172.0
2006	793	115.7	13.6	38.2	167.5
2007	797	110.3	12.9	38.2	161.4
2008	733	96.2	12.3	38.2	146.6
2009	690	85.8	11.6	38.2	135.7
2010	663	78.2	11.0	38.2	127.4
Year	Milkweeds in agricultural fields				Total milkweeds§§
	Total ag hectares**	Mlkwd density††	Total ag mlkws‡‡		
1999	9267	23.00	213.2		398.5
2000	9308	19.75	183.8		370.9
2001	9186	16.92	155.4		350.4
2002	9166	14.55	133.4		324.8
2003	9267	12.49	115.8		300.4
2004	9267	10.73	99.4		277.3
2005	9247	9.21	85.2		257.2
2006	9207	7.91	72.8		240.3
2007	9247	6.79	62.8		224.2
2008	9328	5.83	54.4		201.0
2009	9389	5.00	46.9		182.6
2010	9389	4.29	40.3		167.6

\*×1000; from USDA Conservation Programs (2010).

†m<sup>2</sup> × 1000; CRP ha × 212 m<sup>2</sup> ha<sup>-1</sup> (milkweed density from H&B, 2000) × 0.948<sup>x</sup> (where x = 0 for 1999).

‡m<sup>2</sup> × 1000; 1416 ha (Lubowski *et al.*, 2006) × 14 m<sup>2</sup> ha<sup>-1</sup> (milkweed density from H&B, 2000) × 0.948<sup>x</sup> (where x = 0 for 1999).

§m<sup>2</sup> × 1000; 386 ha (Lubowski *et al.*, 2006) × 99 m<sup>2</sup> ha<sup>-1</sup> (average milkweed density from H&B, 2000 and H, 2010).

¶m<sup>2</sup> × 1000; Conservation Reserve Program (CRP) milkweeds + Pasture milkweeds + Roadside milkweeds.

\*\* × 1000; from Iowa State Ag. Statistics (2010).

††m<sup>2</sup> ha<sup>-1</sup>; 1999 value from H&B (2000), 2009 value from H (2010); others = 1999 value × 0.858<sup>x</sup> where x = 0 for 1999.

‡‡m<sup>2</sup> × 1000; Ag ha × Milkweed density.

§§m<sup>2</sup> × 1000.

acres in roadside and pasture have not changed substantially over the last decade. Data on the acres planted to corn or soybeans by year were obtained from Iowa State Agricultural Statistics (2010) and the amount of Iowa CRP land from the USDA Conservation Programs (2010).

#### *Estimating monarch use of non-agricultural milkweeds*

To estimate monarch use of non-agricultural milkweeds, we used data on the number of monarch eggs per milkweed stem from the MLMP. We examined MLMP data from 1999 to 2010 for sampling localities within the Midwest (eastern Kansas, eastern Nebraska, eastern North and South Dakota, Minnesota, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana and western Ohio). Sites were excluded in any given year if the average number of milk-

weeds monitored was <25 and if there were fewer than five sampling events in July and August. We also excluded garden sites because they represent a minor component of milkweeds on the landscape. Sites were excluded if volunteers observed more larvae than eggs because these volunteers may not have been able to discern monarch eggs accurately. We initially divided sites into two groups based on the habitats in which the milkweeds were found: 'natural areas' (prairies or nature preserves) and 'other' (pastures, old fields, roadsides and CRP land); there were no sites in agricultural fields. However, 'natural areas' and 'other' were not significantly different from each other in egg density and were combined in the analysis into a single 'non-agricultural' category.

For any site, the number of eggs per plant varies over the course of the season. However, there is a population build-up during July and August when the second/third generation

occurs (MLMP, 2011). We used egg density at the peak of this build-up as a metric of annual production. For each year, our estimate of production was based on the average maximum egg density over all MLMP sites. This metric does not include all of the annual production but does allow us to examine the relative differences in production among years.

*Monarch use of milkweeds in agricultural fields*

Pleasants monitored milkweed populations and monarch activity in agricultural fields and non-agricultural habitats in Iowa from 2000 to 2003. Initially six study sites were selected. Each site included a field planted to soybeans, another field adjacent or nearby that was planted to corn and a nearby non-agricultural habitat. Non-agricultural habitats included natural areas, pastures, old fields and roadsides. CRP land was not explicitly included as a habitat type but the non-agricultural habitats selected are similar in vegetative characteristics to CRP land. Sites were all located within a 10 km radius of Ames, Iowa, except for one site located 40 km south of Ames. Over the years of study, a few sites were removed from monitoring for logistical reasons and a few others added but in all years, both agricultural and non-agricultural plots were examined. Within each site, patches of milkweeds were marked (milkweed plots). These patches were relatively discrete units that ranged in area from 3 × 3 to 6 × 10 m and contained 10–150 milkweed stems. In each field, approximately 10 milkweed plots were chosen and mapped using a global positioning system device so they could be relocated in subsequent years. Sites were visited at weekly intervals: in 2000 from late May to late August; in 2001 from early July through late August; and in 2002 and 2003 from early June to late August. During each visit, every milkweed stem in each milkweed plot was inspected for monarch eggs and larvae.

As described above, we used the maximum number of eggs per stem observed during the weekly censuses from July through August as the measure of production. Egg densities in different non-agricultural habitat types were not statistically different, so they were combined into one category. Egg densities on milkweed in corn and soybean fields in any year were not statistically different from each other and were combined into a single cate-

gory. The results are shown in Table 2. Egg densities on milkweeds in agricultural fields were significantly higher than on milkweeds in non-agricultural habitats in each year by an average factor of 3.89.

*Estimating potential monarch production*

Potential monarch production for any year is equal to the sum of egg production from two sources: non-agricultural and agricultural milkweeds. To calculate production from non-agricultural milkweeds, we first determined the number of milkweeds in non-agricultural habitats. This is equal to the area occupied by each habitat type (CRP land, pasture and roadside) multiplied by the density of milkweeds in that habitat (see Table 1). We then multiplied the total number of non-agricultural milkweeds by the average number of eggs per non-agricultural milkweed plant for that year from the MLMP data (see Table 3). To calculate production from agricultural fields, we first determined the number of milkweeds in fields. This is equal to the area occupied by agricultural land multiplied by the milkweed density in fields (see Table 1). The number of agricultural milkweeds in each year was multiplied by the eggs per agricultural milkweed plant. For the years 2000–2003, we used Iowa data for the eggs per agricultural milkweed (from Table 2). For each of the other years, the egg density on agricultural milkweeds was taken to be 3.89 times the MLMP value for that year (see Table 3).

**Results**

Estimates of milkweed numbers on the Iowa landscape (Table 1) show that milkweeds declined in both agricultural fields and non-agricultural habitats from 1999 to 2010. There was a 31% decline for non-agricultural milkweeds and an 81% decline for agricultural milkweeds with a 58% overall decline for total milkweeds. In 1999, milkweeds in agricultural fields constituted 53% of total milkweeds, but by 2010 were only 24% of the total. The 58% loss of milkweeds on the landscape actually underestimates the loss of resource for monarchs, because most

**Table 2.** Maximum eggs per milkweed stem July through August for agricultural and non-agricultural sites in Iowa where ‘*n*’ is the number of fields examined. Egg densities on milkweeds in agricultural fields were significantly higher than on milkweeds in non-agricultural habitats in each year (2000: *t* = 3.97, d.f. = 11; 2001: *t* = 2.90, d.f. = 4; 2002: *t* = 3.35, d.f. = 4; *t* = 4.54, d.f. = 5; all *P*-values < 0.02).

Year	Maximum eggs per milkweed						Ratio ag/non-ag
	Agricultural			Non-agricultural			
	Avg.	SE	<i>n</i>	Avg.	SE	<i>n</i>	
2000	0.796	0.140	10	0.197	0.049	8	4.05
2001	1.661	0.459	5	0.329	0.021	3	5.05
2002	0.659	0.123	4	0.205	0.056	4	3.21
2003	1.125	0.108	5	0.345	0.133	3	3.26
					Average ratio		3.89

**Table 3.** Estimate of egg production in the Midwest from 1999 to 2010. Note that values in the final three columns are relative; egg densities are in eggs/stem whereas milkweed densities are not in stems  $\text{ha}^{-1}$  but  $\text{m}^2 \text{ha}^{-1}$ .

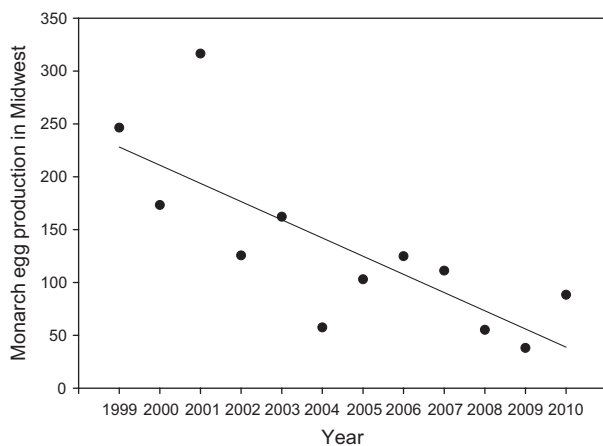
Year	Total non-ag milkweeds*	Total ag milkweeds*	Eggs/plant-non-ag†	Eggs/plant-ag‡	Production non-ag§	Production ag¶	Total production**
1999	185.4	213.2	0.243	0.945	45.0	201.4	246.5
2000	187.1	183.8	0.144	0.796	26.9	146.3	173.2
2001	195.0	155.4	0.299	1.661	58.3	258.2	316.5
2002	191.5	133.4	0.197	0.659	37.6	87.9	125.5
2003	184.7	115.8	0.173	1.125	31.9	130.2	162.1
2004	177.9	99.4	0.102	0.395	18.1	39.3	57.4
2005	172.0	85.2	0.205	0.796	35.2	67.8	103.0
2006	167.5	72.8	0.277	1.077	46.4	78.5	124.9
2007	161.4	62.8	0.274	1.066	44.2	66.9	111.1
2008	146.6	54.4	0.154	0.599	22.6	32.6	55.2
2009	135.7	46.9	0.120	0.465	16.2	21.8	38.0
2010	127.4	40.3	0.311	1.210	39.6	48.7	88.4

\* $\text{m}^2 \times 1000$ ; from Table 1.

†from MLMP.

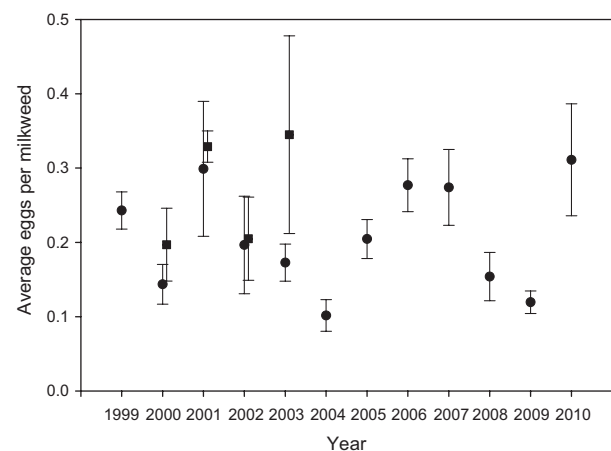
‡Non-ag eggs/plant  $\times 3.89$  (ratio of ag to non-ag, see Table 2), except for 2000–2003 from Table 2.§Total non-ag milkweeds  $\times$  Eggs/plant non-ag.¶Total ag milkweeds  $\times$  Eggs/plant ag.

\*\*Production non-ag. + Production ag.

**Fig. 2.** Estimate of monarch production from the Midwest by year. Linear regression  $F_{1,11} = 13.7$ ;  $P = 0.004$ ;  $r^2 = 0.58$ .

of the loss was in agricultural fields and each agricultural milkweed represents 3.89 times more monarch eggs than a non-agricultural milkweed (Table 2). If the numbers of agricultural milkweeds in Table 1 are multiplied by 3.89 to convert them to their resource potential, the decline in the milkweed resource base is 72%. Of this potential resource lost, 92% comes from agricultural fields and 8% from non-agricultural habitats. Table 3 shows the conversion of yearly milkweed numbers into monarch production. The relative contribution of agricultural milkweeds to total monarch production went from 82% in 1999 to 55% in 2010.

There has been a significant decline in monarch egg production over the last decade (Fig. 2 – linear regression  $F_{1,11} = 13.7$ ,  $P = 0.004$ ,  $r^2 = 0.58$ ). On the basis of regression equation for

**Fig. 3.** Average maximum egg density (eggs per milkweed stem  $\pm 1$  SE) for July through August for non-agricultural milkweeds at sites throughout the Midwest (from MLMP). Number of sites: 1999, 16; 2000, 41; 2001, 13; 2002, 25; 2003, 41; 2004, 46; 2005, 49; 2006, 57; 2007, 29; 2008, 29; 2009, 30; 2010, 21. Square symbols indicate the average value ( $\pm 1$  SE) for non-agricultural sites in Iowa (from Table 2). The Iowa value for each year was not significantly different from the MLMP value.

this decline ( $y = 254.4 - 17.21x$ , where  $x = 1$  when the year is 1999), we estimate that between 1999 and 2010 monarch egg production in the Midwest was reduced 81%. This decline in production would not have occurred if monarchs had increased their use of the remaining milkweeds as agricultural milkweeds declined. However, egg density on non-agricultural milkweeds from the MLMP data did not show a significant change over the years (Fig. 3) (because of non-normality, a Poisson regression was used; Wald  $\chi^2 = 0.15$ ; d.f. = 1;  $n = 398$ , NS). We

also compared our estimate of potential monarch production in each summer with the size of the population that subsequently overwintered in Mexico (Fig. 4). Yearly production values were positively correlated with the size of the overwintering population (linear regression  $F_{1,11} = 8.97$ ,  $P = 0.01$ ,  $r^2 = 0.47$ ).

## Discussion

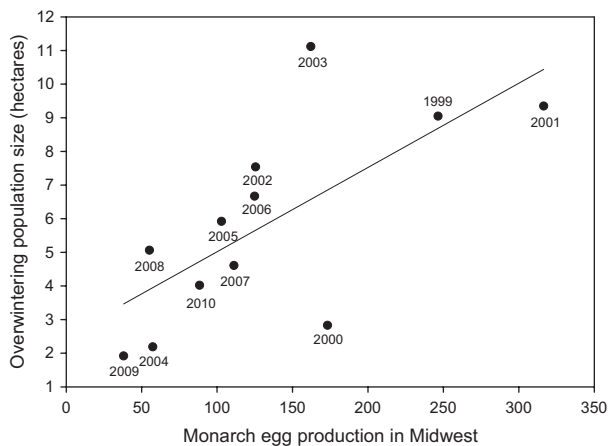
Our estimate of monarch production decline in the Midwest was based in part on Iowa data. To what extent do Iowa data reflect the Midwest as a whole? We used Iowa data to estimate (i) the proportion of milkweed in various habitats, (ii) the density of milkweeds in each habitat, (iii) the decline in milkweeds in agricultural fields and (iv) the relatively higher egg density on agricultural milkweeds compared to non-agricultural milkweeds. We examine each of these aspects of the data. (i) Data on land use for the Midwestern states (Lubowski *et al.*, 2006) show that of the potential milkweed habitat 73% was in crop production and 27% in non-agricultural habitats (6% in CRP land, 6% in cropland pastures, 11% in grassland and range pastures, and 4% in roadsides). This is similar to the 79% in crop production for the state of Iowa and 21% in non-agricultural habitats (6% in CRP land, 5% in cropland pastures, 7% in grassland and range pastures and 3% in roadsides). Note that these values do not include forested land as this is not milkweed habitat. This comparison excluded the Northern Plains states (Kansas, Nebraska, N. and S. Dakota), which have extensive grasslands and rangeland in the western sections. If those states are included, the per cent of Midwest land in crops falls to 60% with 40% of land non-agricultural. (ii) Iowa data were used to estimate milkweed densities for agricultural and roadside habitats; the change in milkweed density in other non-agricultural habitats was based on Midwest MLMP data. There has not been a long-term study of milkweed density in agricultural habitats

outside of Iowa so the similarity between Iowa and the Midwest in this aspect can only be assumed. (iii) Other Midwest areas have seen a decline in milkweed density in agricultural fields over the past decade. Two of the Wisconsin fields originally surveyed by Oberhauser in 2000 (Oberhauser *et al.*, 2001) were resurveyed in subsequent years. In 2000, these sites had an average of 0.28 milkweed stems  $m^{-2}$ , and in 2002–2006, after the growers began to use glyphosate-tolerant soybeans in 2001, no milkweeds were found. (iv) Higher egg densities on agricultural milkweeds were also observed in other states in the Midwest in 2000 (Oberhauser *et al.*, 2001).

Further evidence suggesting that our approach, which combines data from Iowa and Midwest sources, does reflect production for the Midwest as a whole comes from the significant positive correlation between the annual estimate of monarch production and the size of the subsequent overwintering population (Fig. 4). Because the Midwest contributes about half of the individuals to the overwintering population (Wassenaar & Hobson, 1998), we would expect such a correlation if Midwest production were accurately estimated. We note, however, that the estimate of the Midwest contribution to the overwintering population was made before significant glyphosate use in row crops and only represents 1 year of data.

Although our estimates of annual Midwest monarch production were highly correlated with the size of the subsequent overwintering population, these estimates explained only 47% of the variation in the size of the overwintering population. In particular, our production value for 2003 underestimated the overwintering population size and our value for 2000 overestimated it. We suggest four possible reasons for such deviations. (i) Deviations may be due to the fact that we have used egg density as our measure of production, which is a measure of potential production, while actual production is adult butterflies. The relationship between potential and actual production will depend on survivorship from egg to adult, which may vary among years (J. M. Pleasants & K. S. Oberhauser, unpubl. data). (ii) The relative contribution of the Midwest to the population as a whole is likely to vary from year to year (K. S. Oberhauser, unpubl. data). (iii) The amount of mortality during the fall migration is likely to vary among years depending on conditions along the migratory route including nectar availability, temperature, weather events, drought conditions and wind conditions. (iv) We used a factor of 3.89, the average of 4 years of Iowa data, to convert agricultural milkweeds into their monarch egg production. The factor varies among years, as seen in Table 2, and may be somewhat different in other areas of the Midwest.

The differences between years in egg density per stem seen in the MLMP data (Fig. 3) are likely to be caused by factors in addition to the effect of resource availability. The MLMP egg densities we used came from the second and third generation of monarchs. The size of each generation will depend on the size of the previous generation, each of which will be influenced by the prevailing weather conditions during egg laying and larval development (Zalucki & Rochester, 2004). Although the overwintering population begins this sequence, we found no correlation between the size of the overwintering population and monarch production the following summer. This indicates that other factors, principally temperature and weather conditions, can erase



**Fig. 4.** Estimate of monarch production in the Midwest by year (from Table 3) compared with the size of the subsequent overwintering population in that year (from Rendón-Salinas *et al.*, 2011). The size of the overwintering population is measured in hectares covered by butterflies. Linear regression  $F_{1,11} = 8.97$ ;  $P = 0.01$ ;  $r^2 = 0.47$ .



the influence of the starting population. But environmental conditions alone do not govern population size. Even if favourable conditions exist, the potential production of the monarch population is dampened by the loss of milkweeds.

As previously mentioned, the loss of milkweeds in agricultural fields would not have affected total monarch production if monarch use of the remaining milkweeds, both agricultural and non-agricultural, had increased sufficiently. We do not have data on the use of agricultural milkweeds over the last decade but data from the MLMP indicate that there was no increase in use of non-agricultural milkweeds over this period (Fig. 3). In a modelling study, Zalucki and Lammers (2010) found that removing small patches of milkweed from the matrix (the area between larger patches of milkweed) made it harder for monarch females to achieve their egg production potential because of increased search time. In their model, a decrease in milkweed availability in the agricultural crop matrix, such as what would result from herbicide use, could significantly reduce the lifetime number of eggs laid by individual females.

Davis (2011) has suggested that there has been no downward trend in monarch production, based on monitoring data at two sites at which monarchs congregate during the fall migration. The monarchs that appear at these two sites, Peninsula Point in Upper Michigan and Cape May in New Jersey, are migrants from the Upper Peninsula of Michigan and south central Canada, and the Eastern United States, respectively. However, the isotope analysis of (Wassenaar and Hobson (1998) indicates that monarchs from these areas constitute a much smaller portion of the total monarch population than monarchs from the Midwest. Consequently, the lack of decline Davis observed will not reflect the population as a whole. Similar points have also been argued by Brower *et al.* (2011a).

The lack of decline in migrating Eastern monarchs, noted by Davis, further illustrates the connection between glyphosate use in corn and soybean fields and monarch decline. Monarchs from the Northeast and Canada may not be experiencing a decline because they come from areas with less corn and soybean agriculture and thus less milkweed loss because of herbicide use. In 2010, there were 25.1 million soybean hectares and 25.5 million corn hectares in the Midwest but only 0.4 million soybean hectares and 0.7 corn hectares in the Northeast (USDA, National Agricultural Statistics Service, 2011c).

We estimated that monarch production in the Midwest had declined 81% from 1999 to 2010. For comparison, there was a 65% decline in the size of the overwintering population over this same period (Brower *et al.*, 2011b). The similarity of these percentages, and the fact that our estimate of Midwest production is strongly correlated with the size of the overwintering population, clearly show the dominance of Midwest production for the population as a whole. However, the fact that the size of the overwintering population has declined less than the population contribution from the Midwest reflects the mitigating effect of portions of the range of the species that are not dominated by corn and soybean agriculture and have not been impacted by milkweed loss. As the monarch production contribution from the Midwest declines, the relative contribution from other parts of the range increases. A reassessment of the production contribution of the Midwest and other parts of the range, such as that

performed earlier by Wassenaar and Hobson (1998), would be useful.

The loss of milkweeds in agricultural fields is particularly devastating for the monarch population because agricultural milkweeds are more heavily used than non-agricultural milkweeds (Table 2). This difference in egg density could result if females that find patches of milkweeds in agricultural fields lay more eggs per stem or if more females find patches of agricultural milkweeds. Patch size is typically smaller in agricultural fields than in non-agricultural habitats (J. M. Pleasants & K. S. Oberhauser, pers. obs.), and higher egg densities per stem are observed in smaller milkweed patches (Zalucki & Suzuki, 1987). Monarch females may seek out smaller patches and oviposit more heavily there, perhaps because small patches tend to support greater larval success (Zalucki, 1981; Zalucki & Kitching, 1982). Greater oviposition by individual females may also be due to their perception of agricultural milkweeds as being of higher quality. Agricultural milkweed leaves have higher nitrogen content (J. M. Pleasants, unpubl. data) and tend to be in better condition. Finally, the milkweed chemical signal that attracts monarch females may be more apparent against the monoculture background of agricultural fields making it easier for females to find milkweeds in this habitat.

One unexpected finding in this study was the decline in milkweed density in non-agricultural habitats based on measurements by MLMP volunteers. These patches were not chosen at random, and it is possible that this decline is not representative of milkweeds in non-agricultural habitats across the landscape. Milkweed is a disturbance species and as such we would expect colonisation of disturbed areas followed by a population increase for a number of years and then a population decline as milkweed is outcompeted by later successional species. Monitored patches were chosen because they contained high milkweed densities. Thus, they may represent populations that had already experienced some growth and were now in the declining phase. A more thorough survey of milkweed densities in randomly chosen non-agricultural habitats over time is needed. If milkweed densities in non-agricultural habitats are not declining, then the loss of monarch production is not as large as we have estimated. We reran our calculations assuming no decline, and the estimated loss of monarch production from 1999 to 2010 was 76%, somewhat lower than the 81% decline estimated using decreasing milkweed densities in non-agricultural habitats.

Given the disappearance of milkweeds in agricultural fields, milkweeds present in other habitats become more important for monarch populations. Table 1 indicates that the habitat of greatest importance is CRP land. However, the amount of CRP land is also declining; in 2010, the number of CRP hectares for the Midwestern states had declined by 0.5 million from its high in 2007 of 3.8 million hectares (USDA, Conservation Programs, 2010). Farmers have a number of options with regard to what types of vegetation to use as cover on CRP land, with grasses predominating. Adding forbs, including milkweeds, to planting mixes would provide nectar sources that could benefit many insect species and provide host plants for monarchs. While persuading farmers to include milkweed seed in the mix may be difficult, milkweed is capable of colonising such habitats on its own

and education efforts about the value of milkweed and the many non-weedy milkweed species available are underway (Monarch Joint Venture, 2011). Further research needs to be undertaken on CRP land to see how different types of cover vegetation and land management practices affect milkweeds and monarchs.

Roadsides can provide important milkweed habitat; in 2010, 20% of the milkweeds were in roadsides (Table 1), and this value will increase as the remaining agricultural milkweeds disappear. The treatment of roadsides by departments of transportation could influence their value to monarchs. Roadsides are often mowed and sprayed with herbicides to eliminate forbs but roadside management plans compatible with monarch conservation could be developed. Many states are implementing programmes to plant native species along roadsides; such programmes could consider adding milkweeds.

We have not yet seen the full impact that the use of glyphosate herbicides and the consequent reduction in milkweed resources will have on the monarch population. At present, some milkweeds still remain in agricultural fields. Given the established dominance of glyphosate-tolerant crop plants and widespread use of glyphosate herbicide, the virtual disappearance of milkweeds from agricultural fields is inevitable. Thus, the resource base for monarchs in the Midwest will be permanently reduced. This will set a new, lower ceiling for monarch population size. A lower population size could lead to greater vulnerability of the population to deforestation on the overwintering sites and to extreme weather events or climate changes on the overwintering sites, in breeding areas and along migratory routes (Brower *et al.*, 2011b).

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### Author contributions

J.P. performed field studies in Iowa, analysed the data and was primarily responsible for writing the article. K.O. supervised the collection and collation of the data from the MLMP and participated in the writing of the article.

### Competing financial interests

The authors declare no competing financial interests.

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United States Department of Agriculture  
Natural Resources Conservation Service



MONARCH  
JOINT VENTURE

Pollinator Plants of the Central United States

# Native Milkweeds

(*Asclepias* spp.)



June 2013

The Xerces Society for  
Invertebrate Conservation

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# Acknowledgements

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## Photographs

We thank the photographers who generously allowed use of their images. Copyright of all photographs remains with the photographers.

Cover main: Butterfly milkweed (*Asclepias tuberosa*) with black and gold bumble bee (*Bombus auricomus*). Sarah Foltz Jordan, The Xerces Society

Cover bottom left: Monarch caterpillar (*Danaus plexippus*). William M. Ciesla, Forest Health Management International, Bugwood.org

Cover bottom right: Green antelopehorn milkweed (*Asclepias viridis*). Marion Doss.

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## The Xerces Society for Invertebrate Conservation

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# Pollinator Plants of the Central United States

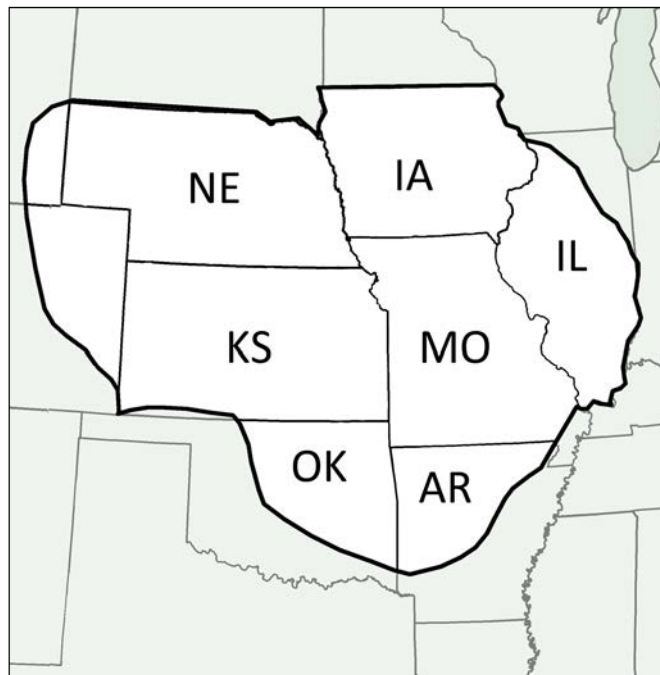
## Native Milkweeds (*Asclepias* spp.)

### Purpose

Milkweeds are native perennial wildflowers that support diverse invertebrate wildlife, including agricultural pollinators such as wild native bees and honey bees. This document describes the plants' value to wildlife, highlights NRCS conservation practices in which milkweeds can be included, provides an overview of milkweed establishment practices, and profiles numerous species that are commercially available and can be incorporated into seed mixes and planting plans. Milkweeds that have special conservation status are also profiled.

### Introduction

Milkweeds (*Asclepias* spp.) occur in a diversity of plant communities in the central United States including prairies, meadows, glades, open woods, forest margins, wetlands, and riparian areas. Milkweeds are the required larval host plants for the monarch butterfly (*Danaus plexippus*), and support a tremendous range of pollinators and other insects with abundant, high quality nectar. Milkweed flower visitors include native bees, honey bees, butterflies, beetles, flies, and hummingbirds. Milkweeds are named for their milky sap, which contains latex and complex chemicals (cardenolides) that make the plants unpalatable to most animals. The plants have fleshy, pod-like fruits (follicles) that split when mature, releasing the seeds. White, fluffy hairs (the pappus), are attached to each seed. These hairs, also called coma, floss, or silk, facilitate wind dispersal of the seed.



The central United States.

Blank map image sourced from Wikimedia Commons, with permission to use and modify it granted through a GNU General Public License.



Photo: © iStockphoto.com/ImagesbyDebraLee

Milkweed seed dispersing from a mature pod.

Milkweeds have a variety of ethnobotanical uses. Native Americans used stem fibers to make string, rope, and cloth (Stevens 2001). Also, some tribes used common milkweed (*Asclepias syriaca*) sap to remove warts and to treat ringworm and bee stings (Stevens 2000). Butterfly milkweed (*A. tuberosa*) roots, called "pleurisy root" have many medicinal uses, such as relieving inflammation of the lungs (Stevens 2001). Pleurisy root is currently sold as an over-the-counter herbal medicine. During World War II, millions of pounds of milkweed floss were used to fill life preservers and other life-saving equipment (Berkman 1949). Milkweed floss is currently used as hypo-allergenic filling for pillows and comforters.

Milkweeds were historically common and highly diverse in the tallgrass prairies that once dominated the central United States. However, milkweeds and many other native plant species have been eradicated on a broad scale, with agricultural intensification and the widespread adoption of herbicide resistant crops as significant drivers of habitat loss (Brower et al. 2012; Pleasants and Oberhauser 2012; Wilcove et al. 1998). At present, five milkweed species that occur in the region are federally or state listed as threatened or endangered (PLANTS Database). Despite milkweeds' native status and value to the monarch butterfly and a diversity of other pollinators, they are sometimes perceived as weeds and are eradicated from agricultural fields, pastures, and roadsides. While some milkweed species colonize lightly disturbed areas, most require specific habitat conditions and with the exception of common milkweed, do not often occur in cultivated cropland.

The scope of this document covers the Lower Midwest and central United States: Nebraska, Iowa, the majority of Kansas, Missouri, and Illinois, and significant parts of Oklahoma and Arkansas (map at left). Thirty milkweed species occur within this region, which is characterized by the eastern Great Plains in the west, the Ozark Mountains in the central part of this region, and the tallgrass prairie and central Mississippi River valley in the east.



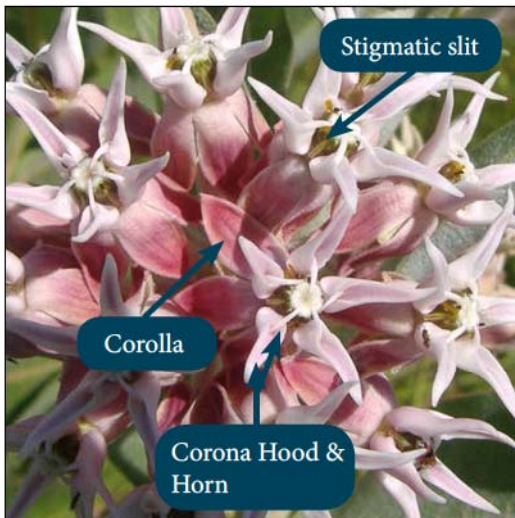


Photo: Eric Eldredge, USDA-NRCS.

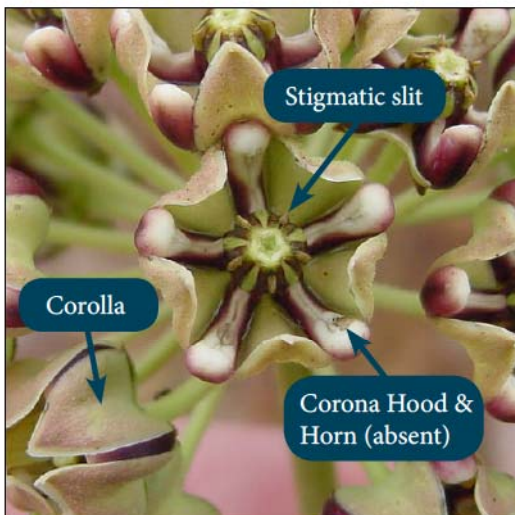


Photo: Linda Kennedy, National Audubon Society.

The structure of milkweed flowers remains consistent, despite one species appearing quite different from another. At the top is showy milkweed (*Asclepias speciosa*); at the bottom is antelope horns (*A. asperula*).

### Toxicity to Livestock

The chemicals contained in milkweeds make them potentially toxic to livestock and other mammals. Milkweeds are distasteful to livestock (Kingsbury 1964) and animals do not usually eat them if sufficient forage is available (USDA ARS 2006). Most livestock losses result from hungry animals being concentrated in areas where milkweed is abundant (USDA ARS, 2006). Symptoms of poisoning include profound depression, weakness, labored breathing, and seizures (Burrows and Tyrl 2006). Poisoning may also occur if animals are fed hay containing large amounts of milkweed (USDA ARS, 2006); it is important to avoid the inclusion of milkweed in prepared feeds and hay. For more information about toxic dosage and signs of potential poisoning to livestock from consuming milkweeds, see Kingsbury (1964) and Burrows and Tyrl (2006).

### Milkweed Pollination

Milkweed flowers have a unique shape and are pollinated in a more specific way than most other insect-visited flowers. Rather than occurring as individual pollen grains that cling to a flower visitor, milkweed pollen is contained in pairs of tiny, waxy sacs (pollinia), located in vertical grooves (stigmatic slits) of the flowers. When an insect visits a flower to sip nectar, its legs or mouthparts may slip into one of the grooves, thereby attaching pollinia to the insect. Pollination occurs when pollinia are inadvertently transferred by the insect into another milkweed flower. See Bookman, 1981, for a more complete discussion of milkweed floral morphology and terminology, and Morse, 1982, for more information on pollinia transfer by insects.

### Monarch Butterflies

Milkweeds are the required host plants for monarch caterpillars. Female monarchs lay their eggs on milkweeds and once hatched, caterpillars feed on the plants for up to two weeks before forming chrysalises. Both caterpillars and adult butterflies store the plants' distasteful chemicals in their bodies, giving them some protection from predators.

Monarchs' annual migration in North America is a widely-known phenomenon. During spring, summer, and early fall, monarchs breed throughout the United States and southern Canada, and several generations of butterflies are produced each year. Butterflies of the last generation born in late summer or early fall migrate to either central Mexico or the California coast to spend the winter congregated in forest habitats. Though exceptions have been documented, fall monarchs produced east of the Rocky Mountains typically migrate to Mexico, while monarchs produced west of the Rockies typically migrate to coastal California. Tens of millions of butterflies overwinter in Mexico, whereas hundreds of thousands overwinter in California. In late winter and early spring, monarchs depart from overwintering sites in search of milkweeds on which to lay their eggs, thus starting the annual cycle again.

Unfortunately, the future of the monarch migration is at risk. Annual monitoring of overwintering monarchs has revealed significant population declines. For example, over the last 19 years, the average forested area occupied by monarchs in central Mexico was 6.69 hectares (16.53 acres). From 2004–2013, the estimated occupied area has been below average each year, with the 2012–2013 population being 1.19 ha (2.94 ac), the smallest estimated since comprehensive monitoring began in 1994 (Rendón-Salinas and Tavera-Alonso 2013). In addition to severe weather and forest degradation in the overwintering areas, a major factor contributing to these population declines is believed to be the loss of milkweed plants from the monarch's breeding range (Brower et al. 2012; Pleasants and Oberhauser 2012; Commission for Environmental Cooperation 2008).

The central United States is an important monarch breeding area and monarchs are typically present in the region throughout the spring and summer. In the mid-1990s, stable-isotope research showed that approximately 50% of the fall migrants arriving in Mexico were produced in the north central United States (Wassenaar and Hobson 1998). However, steep declines of milkweeds and monarchs have resulted from the widespread adoption of herbicide-resistant corn and soybean crops across the region. Prior to the introduction of these crop varieties that are genetically modified to tolerate application of glyphosate-based herbicides, common milkweed (*Asclepias syriaca*) was widespread along the margins of row crop fields. Between 1999 and 2009, Hartzler (2010) documented a 90% decline of common milkweed in Iowa corn and soybean fields. Pleasants and Oberhauser (2012) estimated a 58% decline of milkweed density in the Midwest land-



scape between 1999 and 2010, with a corresponding 81% decline in monarch production.

These documented declines in milkweed habitat and monarch breeding potential illustrate the urgent need to protect existing milkweed populations and also to increase the abundance of milkweeds through restoration activities.

### Agricultural Pollinators

Native plants such as milkweeds play an important role in supporting both wild bees and honey bees. Wild native bees provide free pollination services, and contribute an estimated \$3 billion worth of crop pollination annually to the U.S. economy (Losey and Vaughan 2006). However, these resident pollinators are active in the field longer than the duration of a crop's bloom period, and require sources of pollen and nectar throughout spring, summer, and fall. Research has shown that managed honey bees are healthier and more resistant to diseases when they have access to diverse and abundant floral resources (Alaux et al. 2010). The International Bee Research Association classifies North American milkweeds as class 1/2 for honey production, reflecting a theoretical yield of up to 50 kilograms of honey produced per hectare of milkweed plants (i.e., if they occurred as a contiguous stand) (Ramsay 1987).

### Supporting Beneficial Insects

In addition to attracting pollinators, milkweeds support insects that are natural predators and parasitoids of many crop

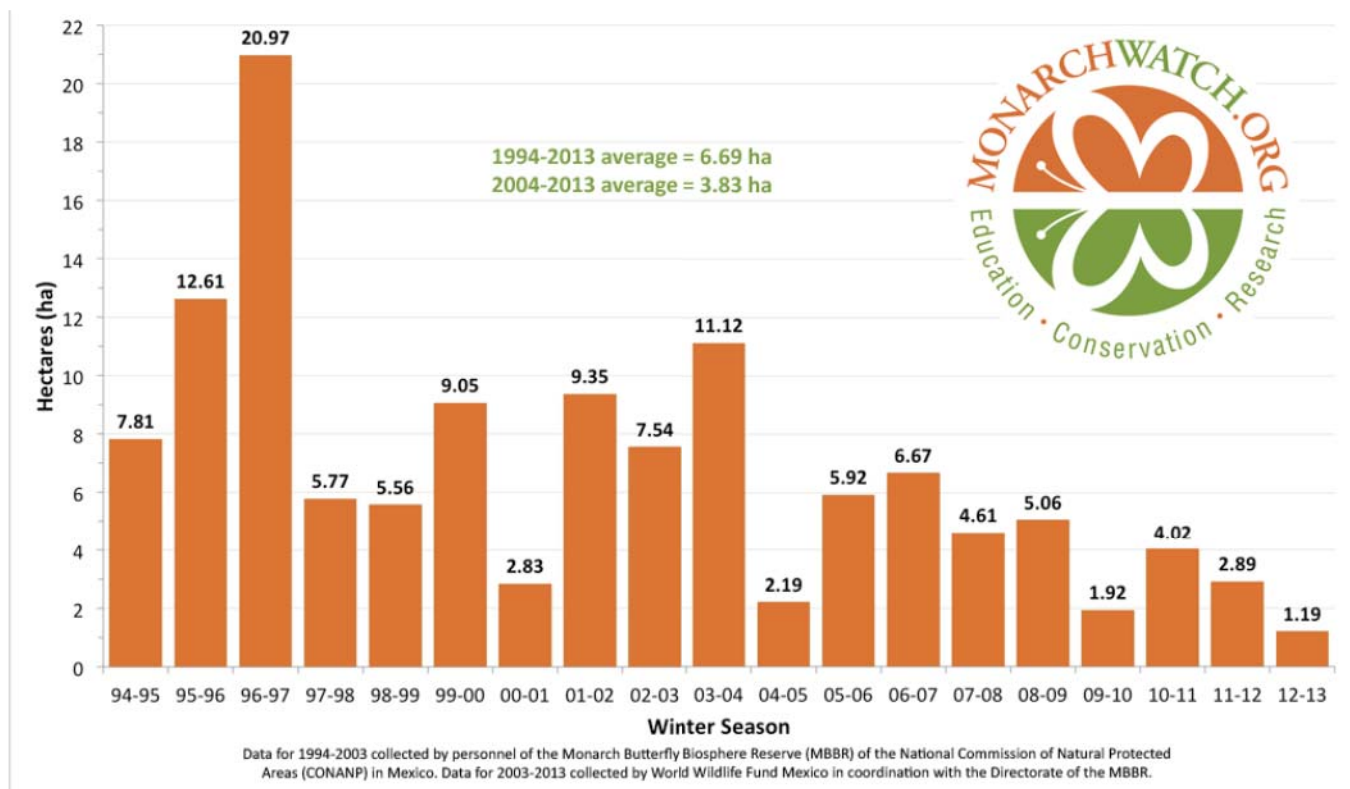
and garden pests. Many wasps, beetles, and flies, which are important groups of beneficial insects, have relatively short tongues and preferentially forage on flowers such as milkweeds that have easily accessible nectar (Mader et al. 2011).

A recent study conducted in Washington state evaluated 43 species of native flowering perennials for their potential to attract beneficial insects. Showy milkweed (*Asclepias speciosa*) attracted the most beneficial insects of any plant species studied, including mite-eating ladybeetles (*Stethorus* spp.), minute pirate bugs (*Orius* spp.), hover flies (Syrphidae), and parasitic wasps (Ichneumonidae, Braconidae) (David G. James, unpublished data). In Michigan, swamp milkweed (*Asclepias incarnata*) has been documented as very attractive to natural enemies of crop pests (Fiedler et al. 2007).

If similar studies of native plant attractiveness to beneficial insects are initiated in this region, it would be advantageous to include one or more milkweeds in the suite of species that are evaluated.

### Milkweed Herbivores

In addition to monarch butterflies, insects that are specialized to feed on milkweed include seed bugs (*Lygaeus kalmii*, *Oncopeltus* spp.), longhorn beetles (*Tetraopes* spp.) that feed on foliage and roots, a stem weevil (*Rhyssomatus lineaticollis*), the oleander aphid (*Aphis nerii*), and several additional species of Lepidoptera. These insects are host specific and are not a threat to agricultural crops.



Total area occupied by monarch colonies at overwintering sites in Mexico, between 1994 and 2013. Graph created by Monarch Watch.

# Using Milkweeds in Pollinator Habitat Restoration

## Incorporating Milkweeds into NRCS Conservation Practices

Beginning in 2008, the Farm Bill included language that makes pollinators and their habitat a priority for every USDA land manager and conservationist. For detailed information on how Farm Bill programs can be used to conserve and create habitat for pollinators, please consult the National Technical Note 78 (2008) “Using Farm Bill Programs for Pollinator Conservation”.

Many NRCS conservation practices can be used to create,

enhance, or manage habitat for pollinators and other beneficial insects. Including milkweeds in seed mixes and planting plans will provide both an important source of nectar from late spring through summer (depending on the species planted), and a larval food source for the monarch butterfly. The table below features examples of practices in which milkweeds can be included; it is not an exhaustive list. Each species in the table is profiled later in this document.

Conservation Practice	Code	Recommended Species	Notes
Channel Bank Vegetation	322	ASSP, ASSU, ASSY	Species adapted to moist soils can be included in plantings for both bank stabilization and wildlife value.
Conservation Cover	327	ASAS, ASHI, ASSP, ASSU, ASSY, ASVE, ASTU, ASVI2	Milkweeds are ideal for including in wildflower meadow plantings that are designed to provide habitat for bees.
Constructed Wetland	656	ASIN	Swamp milkweed is a wetland-adapted species that is common in the region.
Critical Area Planting	342	ASAS, ASVE	These species grow well on slopes and hillsides, and may be useful for soil stabilization.
Early Successional Habitat Development/Management	647	ASAS, ASHI, ASSP, ASSU, ASSY, ASVE, ASTU, ASVI2	Several milkweeds have good colonizing ability and thrive in open habitats with full sun exposure.
Field Border	386	ASAS, ASHI, ASSP, ASSU, ASSY, ASVE, ASTU, ASVI2	Field borders provide numerous conservation benefits beyond supporting pollinators. Use caution where spread by underground rhizomes is undesirable.
Hedgerow Planting	422	ASHI, ASSP, ASSU, ASSY, ASVE	Milkweeds can be incorporated into hedgerow edges, providing additional plant structure. Use caution where spread by underground rhizomes is undesirable.
Pest Management	595	ASAS, ASHI, ASSP, ASSU, ASSY, ASVE, ASTU, ASVI2	Some studies have shown that milkweed nectar is highly attractive to beneficial insects that prey upon or parasitize pest insects. Providing habitat for such insects has been demonstrated to be valuable for vineyards, orchards, and other crops.
Riparian Herbaceous Cover	390	ASSP, ASSU, ASSY	Species adapted to moist soils and high water tables can provide resources for monarchs and pollinators using riparian areas.
Streambank and Shoreline Protection	580	ASIN	Swamp milkweed can provide bank and shoreline protection as well as nectar for flower visitors.
Wetland Enhancement	659	ASIN	Where forb enhancement is desired, swamp milkweed can be included.
Wetland Restoration	657	ASIN	Swamp milkweed can be used when restoration plantings include a forb component.

Scientific and common names, with milkweed abbreviated as “mw”: ASAS: *A. asperula* (antelope horns); ASHI: *A. hirtella* (tall green mw); ASIN: *A. incarnata* (swamp mw); ASSP: *A. speciosa* (showy mw); ASSU: *A. sullivantii* (prairie mw); ASSY: *A. syriaca* (common mw); ASTU: *A. tuberosa* (butterfly mw); ASVE: *A. verticillata* (whorled mw); ASVI2: *A. viridis* (green antelopehorn).



### Protecting Existing Milkweed Stands

Where milkweeds already occur, in remnant natural areas or non-cropped areas such as field borders, roadsides, and ditch banks, conservation practices that involve the management of existing habitat can protect the plants as a resource for monarch caterpillars, pollinators, and other beneficial insects. It is ideal to leave milkweeds undisturbed to the greatest extent

possible throughout the growing season, especially when they are flowering. Milkweeds can potentially host monarch caterpillars whenever the plants have foliage. If maintenance activities such as mowing, spraying, or burning must be conducted during the growing season, it is ideal to treat only a subset of the total area occupied by milkweed. Many of the species featured in the table below are profiled later in this document.

Conservation Practice	Code	Recommended Species	Notes
Restoration and Management of Rare and Declining Habitats	643	ASAM, ASAR, ASAS, ASEN, ASHI, ASIN, ASLA3, ASLA4, ASME, ASOV, ASPU2, ASSP, ASST, ASSU, ASSY, ASVE, ASTU, ASVI, ASVI2	Preventing tree and shrub encroachment, and controlling non-native grass invasion can maintain or enhance the habitat quality of remnant prairies in which numerous milkweeds potentially grow. The majority of milkweeds native to the region, including those with special conservation status, are associated with prairie communities.
Upland Wildlife Habitat Management	645	All species except for swamp milkweed, which is wetland-adapted.	Practices such as livestock grazing and controlling invasive plant species will promote native plant diversity, thus providing diverse resources for wildlife.
Wetland Wildlife Habitat Management	644	ASIN	Where applicable, eradicating invasive plant species will encourage native plant diversity. Minimizing or avoiding pesticide use will promote beneficial insect health.

Scientific and common names, with milkweed abbreviated as “mw”: ASAM: *A. amplexicaulis* (clasping mw); ASAR: *A. arenaria* (sand mw); ASAS: *A. asperula* (antelope horns); ASEN: *A. engelmanniana* (Engelmann’s mw); ASHI: *A. hirtella* (tall green mw); ASIN: *A. incarnata* (swamp mw); ASLA3: *A. lanuginosa* (sidecluster mw); ASLA4: *A. latifolia* (broadleaf mw); ASME: *A. meadii* (Mead’s mw); ASOV: *A. ovalifolia* (oval-leaf mw); ASPU2: *A. purpurascens* (purple mw); ASSP: *A. speciosa* (showy mw); ASST: *A. stenophylla* (slimleaf mw); ASSU: *A. sullivantii* (prairie mw); ASSY: *A. syriaca* (common mw); ASTU: *A. tuberosa* (butterfly mw); ASVE: *A. verticillata* (whorled mw); ASVI2: *A. viridis* (green antelopehorn); ASVI: *A. viridiflora* (green comet mw).

## Milkweed Establishment

Milkweeds are most easily established from seed, and germination rates are typically high. Transplants (plugs) of some species are also commercially available. Milkweeds prefer full sun and most will tolerate dry soil conditions. Native milkweeds of this region are deciduous perennials. Following seed dispersal, they die back to the ground. They remain dormant during the winter, and re-emerge in the spring from established root systems. With the exception of prolonged drought, the plants will not require any supplemental watering.

### Site Preparation and Seeding

Milkweed seed should ideally be planted in the fall. While some seed predation will occur, exposure to cold temperatures and moist conditions during winter will stimulate germination. Spring planting is also possible but artificial stratification of the seed is recommended to enhance germination. For planting areas several acres in size, milkweed can be included in native seed mixes and direct seeded to a depth of ¼–½ inch, using a specialty wildflower seed drill. Direct seeding requires good soil preparation; the seedbed should be a smooth, lightly packed surface, free of clumped sod and plant debris. For small sites, a rake or turf roller can be used to remove or break up large dirt clods. For large areas, the soil can be prepared with a cultipacker, spike tooth harrow, or similar tractor-drawn equipment. For planting areas of any size, seed can

also be broadcast onto a smooth, weed-free soil surface. To achieve good seed to soil contact, the seed can be compacted into the ground with a cultipacker, lawn roller, or the wheels of an ATV or tractor. To facilitate milkweed establishment, it is important to eradicate existing weed cover and deplete the amount of weed seeds in the soil seed bank. Depending on the abundance of weeds or weed seed at the planting site, one to two full years of weed control may be needed. Weeds can be controlled through tillage, herbicide application, flaming, smothering, or a combination of those methods. For more detailed information on site preparation and seeding, please consult the Job Sheet Installation Guide “Conservation Cover (327) for Pollinators, Upper Midwest” (<http://www.xerces.org/pollinator-conservation/agriculture/pollinator-habitat-installation-guides/>).

### Seedling Propagation and Transplanting

When producing transplants from seed, sowing the seeds in a greenhouse during early February is recommended. Once seedlings have reached desirable size and vigor for transplanting, it is ideal to move them from the greenhouse to a shadehouse, to allow them to acclimate to outdoor environmental conditions for a few days before being transplanted into the field. Feedback from restoration specialists suggests that milkweed seedlings are intolerant



of root disturbance. Transplant shock can be minimized by propagating seedlings in biodegradable peat pots, which can be planted directly into the ground. Transplanting should only be done after the threat of frost has passed. Transplants will need to be watered regularly during the first growing season and weed control may be necessary. Mulching around the plants will help suppress weed growth and reduce water loss.

### Seed Collection and Processing

Milkweed seed can be collected and cleaned by hand when small volumes are needed to create new habitat. It is advisable to avoid collecting pods on which milkweed bugs (*Lygaeus kalmii* and *Oncopeltus* spp.) are present, because their feeding activity renders seeds inviable. Mature milkweed pods split open along a vertical seam and seeds are brown when mature. It is ideal to collect pods when the seam has just begun to split, but before the pod has fully opened and the floss has expanded. When seed will be cleaned by hand, it is easiest to

separate the seeds from the floss at this stage. If pods do not open easily when gently squeezed, the seeds inside will not be mature. If it is not feasible to regularly check plants for pod maturation, mesh “seed capture bags” can be affixed over the maturing pods and to retrieved at a later date. However, if seed will be cleaned by hand, this approach increases the amount of time and labor needed to separate seeds from floss.

Shop vacuums can be an effective tool for cleaning up to a few pounds of milkweed pods. As small handfuls of floss and seed are slowly fed into a vacuum, the lightweight floss fibers tend to aggregate around the filter while the seeds fall into the vacuum receptacle. Minimizing the amount of pod shells that enter the vacuum will result in a cleaner seed lot and reduce the need for further hand cleaning. Because there is considerable range in the design and horsepower of shop vacuums, it is advisable to conduct a small test run to ensure that seeds do not get broken.



Photo: Desert Botanical Garden

A seed capture bag made from lightweight polypropylene garden fabric (“floating row cover”) and affixed with a length of fine-gauge wire. Alternatively, mesh bags with drawstrings can be purchased from some companies that sell corn shoot bags.



Photo: Brianna Borders, The Xerces Society

In our experience, a 5.5 HP shop vacuum was very effective at separating seeds from floss. The floss fibers aggregated around the filter and could be easily removed, while the seeds accumulated in the vacuum receptacle (red canister at right).

## Seed Sourcing

When using milkweeds in habitat restoration efforts, it is important to use wild-type plant materials that are as locally sourced as possible. While the genetic effects of cultivar selection are largely undocumented, plants bred for flower color traits may have lost some of their ability to produce nectar or may have altered chemical composition and therefore may not provide the same resources for wildlife as compared to wild-type plants. Though cultivars are suitable for planting in gardens or urban areas, it is best not to plant them in or near natural areas where they could breed with wild populations. Butterfly milkweed (e.g., ‘Hello Yellow’) and swamp milkweed (e.g., ‘Cinderella’ and ‘Ice Ballet’) are examples of milkweeds

for which selected cultivars are available.

Milkweed seed can be purchased on the internet from multiple vendors but given some species’ broad distribution across the United States, available seed may be of non-local origin. To identify sources of regionally appropriate seed, please ask prospective vendors for details about seed origin. An appendix to this document features a list of vendors that typically have milkweed seed in stock. Some of these vendors also sell milkweed transplants. Additionally, source-identified transplants can be ordered from Monarch Watch ([www.monarchwatch.org](http://www.monarchwatch.org); 888-824-4464).



# Species Profiles

## Commercially Available Species

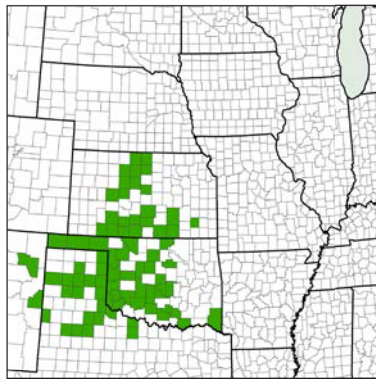
Of the 30 milkweed species that occur in the region, nine are commercially available (to varying degrees) and can be utilized in restoration and revegetation efforts within their native ranges. These species are profiled in the following pages. Seed of butterfly, common, and swamp milkweed is reliably

available by the pound; these species are the most suitable for use in large-scale plantings. Seed of the other six species is often available by the ounce, though supplies may fluctuate from year-to-year. These species can be included in a variety of small-scale plantings.

## *Asclepias asperula* ssp. *capricornu* Antelope horns



Photo: Nancy Lee Adamson, The Xerces Society



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.



Photo: Native American Seed

**Description:** Plants are generally low-growing with multiple stems and sometimes form dense clumps. Corollas form a cup shape below the hoods of the flowers. Leaves are long and usually folded lengthwise. The species' common name is derived from the curved shape of its fruits. Antelope horns is more abundant in Texas (the southern part of its range) than in Oklahoma, Kansas, and Nebraska. Another subspecies, *asperula*, occurs in the western United States. Antelope horns is primarily a spring host plant for the monarch, but depending on summer rainfall received, the plants sometimes have foliage during the fall.

**Flower color:** Corolla light green, corona purple tipped with white

**Bloom time:** May – June

**Maximum height:** 3 feet

**Estimated seeds per pound:** 98,000

**Habitat:** Upland prairie pastures and hillsides, grasslands, and roadside rights-of-way. Grows in many soil types including rocky limestone, gravel, sand, and clay.

### About the Species Profiles

Habitat descriptions in these profiles are derived from University of Kansas herbarium records and numerous regional floras and field guides that are listed in the References and Additional Resources section. Information about bloom time and plant height is also sourced from regional floras and field guides. The range maps showing county-level distribution were created by the USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team,

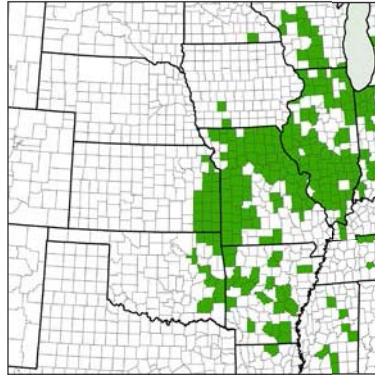
using the latest data available from the PLANTS database. For additional details on species distribution, please consult regional floras. Data on seeds per pound is sourced from the Kew Botanic Gardens Seed Information Database and various seed companies (Cardno JFNew, Everwilde Farms, Prairie Moon Nursery, Roundstone Seed). When multiple data points were available, the figure shown represents an average.



## ***Asclepias hirtella*** Tall green milkweed



Photo: Tom Barnes, University of Kentucky



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Description:** Stems are stout. Leaves are long and narrow, mostly alternate, and hairy. There are typically multiple flower clusters per plant. Within the target region, this species is most abundant in Missouri.

**Flower color:** Corolla green, often tinged with purple; corona green, cream, or lavender

**Bloom time:** May – August

**Maximum height:** 3 feet

**Estimated seeds per pound:** 64,300

**Habitat:** Upland tallgrass prairie, lowland prairie, prairie hay meadows, glades, along roadsides, sometimes in marshy areas. Grows in rocky, sandy, or clay soils.

## ***Asclepias incarnata* ssp. *incarnata*** Swamp milkweed



Photo: David Cappaert, Michigan State University, Bugwood.org



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Description:** Swamp milkweed prefers wet to consistently moist soil, but has some tolerance for drier soil conditions. It can be incorporated into a variety of wetland enhancement and restoration projects. Stems are smooth and usually branched. Leaves are numerous, opposite, and narrow. Another subspecies, *pulchra*, occurs in Texas and several eastern states but not in the central United States. Swamp milkweed is frequently used by monarchs as a host plant.

**Flower color:** Corolla bright pink, corona light pink to white

**Bloom time:** June – September

**Maximum height:** 5 feet

**Estimated seeds per pound:** 86,800

**Habitat:** Wet areas of meadows and prairies, in sloughs and roadside ditches, along the borders of swamps, marshes, ponds, and lakes, along the edges of streams and rivers.



Photo: MJ Hatfield



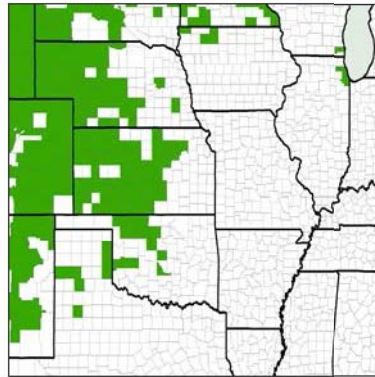
## *Asclepias speciosa* Showy milkweed



Photo: MJ Hatfield



Photo: MJ Hatfield



USDA NRCS PLANTS Database  
Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Habitat:** Moist sandy, loamy, or rocky soils of short grass prairies and roadsides and along rivers, streams, sloughs, ponds, and lake margins.

**Description:** Showy milkweed has the largest flowers of any milkweed. The hoods of the flowers are elongated and together form the shape of a 5-point star. Plants are tall with stout stems and large, broad leaves. Soft hairs cover both the stems and leaves. Showy milkweed is the most widespread, abundant milkweed in the western states. Its range extends into the central United States with numerous occurrences in Oklahoma, Kansas, and Nebraska. Showy milkweed and common milkweed have similar stature and where the species' ranges overlap, it may be difficult to distinguish them when plants are not flowering. The two species are closely related and they hybridize occasionally (Woodson 1954). Showy milkweed sometimes reproduces clonally, through the spreading of underground rhizomes.

**Flower color:** Corolla pink, corona pink or white

**Bloom time:** May – August

**Maximum height:** 4 feet

**Estimated seeds per pound:** 75,000

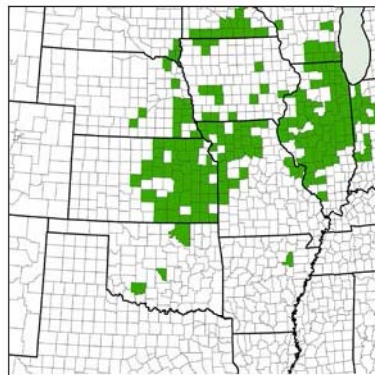
## *Asclepias sullivantii* Prairie milkweed



Photo: R.W. Smith, Lady Bird Johnson Wildflower Center



Photo: R.W. Smith, Lady Bird Johnson Wildflower Center



USDA NRCS PLANTS Database  
Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Habitat:** Sandy, loamy, or rocky calcareous soils of lowland and upland prairies, wet meadows, creek banks, and river bottoms. Also grows along roadsides and railways.

**Description:** Plants are sturdy with thick stems. Leaves are opposite and broad and typically ascend at a pronounced angle. As compared to showy and common milkweed, this species' stems, leaves, and fruits are hairless. Prairie milkweed flowers are larger than those of common milkweed but smaller than showy milkweed flowers. This species reproduces clonally, through the spreading of underground rhizomes.

**Flower color:** Corolla and corona both medium pink, the corona usually of a lighter shade

**Bloom time:** June – August

**Maximum height:** 4 feet

**Estimated seeds per pound:** 84,500



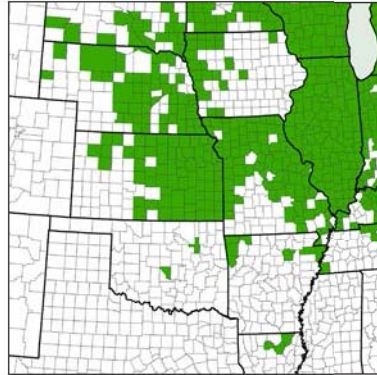
## *Asclepias syriaca* Common milkweed



Photo: Mike Halverson



Photo: Steven Katovich, USDA Forest Service, Bugwood.org



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Habitat:** Prairies, old fields, and margins of woods, in the flood plains of lakes, ponds, or waterways, and along creek banks, roadsides, and railways. Grows in sandy, clay, or rocky calcareous soils.

**Description:** Plants are tall with stout stems and broad, thick leaves. Flowers are fragrant and are borne in multiple clusters per plant. Common milkweed spreads clonally, via underground rhizomes. Common milkweed is the most abundant milkweed in the midwestern and northeastern United States and the most frequently used host plant in the eastern monarch's breeding range. Laboratory analyses of monarch tissues have revealed that the majority of butterflies overwintering in Mexico fed on common milkweed as caterpillars (Malcolm et al. 1993; Seiber et al. 1986). The loss of this important host plant from agricultural fields in the Midwest is likely having a significant impact on monarch breeding potential in the region (Brower et al. 2012; Pleasants and Oberhauser 2012). Flower color: Corolla and corona both a muted pink, the corona usually of a lighter shade.

**Bloom time:** May – August

**Maximum height:** 7 feet

**Estimated seeds per pound:** 61,700

## *Asclepias tuberosa* ssp. *interior* Butterfly milkweed

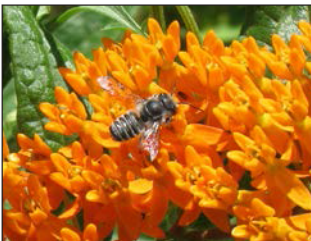
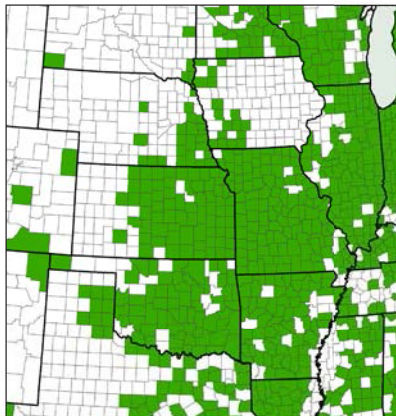


Photo: Mike Halverson



Photo: Allen Casey, Missouri NRCS



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Habitat:**

Well-drained sandy, loamy, or rocky soils in open woods, prairies, savannas, old fields, and along roadsides.

**Description:** Drought-tolerant, broadly adaptable, and one of the most common and

widely distributed milkweeds in the Ozarks, southern Great Plains, and the central Midwest. Another subspecies, *rolfsii*, occurs in the southeastern United States. It is the only milkweed species lacking the milky sap that is characteristic of the genus. Plants typically have multiple stems and often have a bushy appearance. Stems are covered in short, soft hairs. Leaves are also hairy, particularly on the undersides. Butterfly milkweed is an important source of nectar for many insects, but may be a sub-optimal larval host plant for monarchs. Though monarch caterpillars can complete their development on butterfly milkweed, anecdotal evidence and preliminary studies suggest that female monarchs do not prefer to lay eggs on the species (Chip Taylor, Monarch Watch, personal observations).

**Flower color:** Variable, ranging from yellow to orange to red. Most typically, the corolla and corona are both bright orange.

**Bloom time:** May – September

**Maximum height:** 3 feet

**Estimated seeds per pound:** 69,600



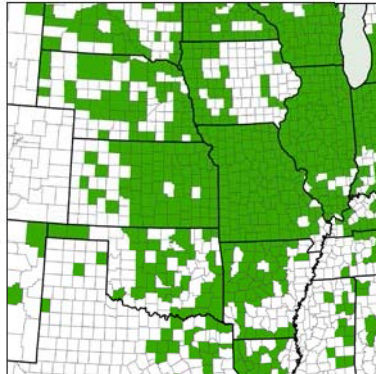
## *Asclepias verticillata* Whorled milkweed



Photo: Chris Evans, Illinois Wildlife Action Plan, Bugwood.org



Photo: Chris Evans, Illinois Wildlife Action Plan, Bugwood.org



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Habitat:** Sandy, clayey, or rocky calcareous soils of prairies, glades, dry open woods, fields, flood plains, and hillsides.

**Description:** Plants are slender and the leaves are narrow, linear, and typically arranged in a whorled pattern around the stem. This species reproduces clonally, through the spreading of underground rhizomes. Whorled milkweed is one of the more toxic milkweed species; livestock managers should take appropriate measures to prevent animals from consuming the plants.

**Flower color:** Corolla white, green, or brown; corona white

**Bloom time:** June – September

**Maximum height:** 3 feet

**Estimated seeds per pound:** 182,000

## *Asclepias viridis* Green antelopehorn

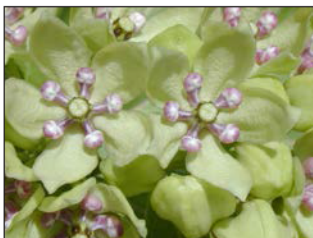
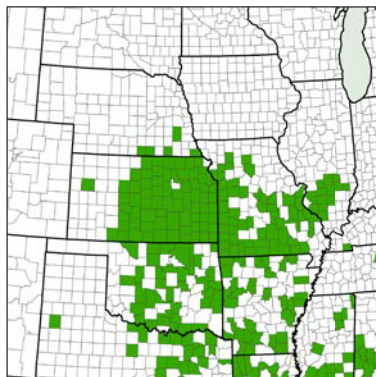


Photo: Joseph A. Marcus, Lady Bird Johnson Wildflower Center



Photo: John D. Byrd, Mississippi State University, Bugwood.org



USDA NRCS PLANTS Database

Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Habitat:** Sandy or rocky soil in prairies, glades, grasslands, and pastures, and along roadsides.

**Description:** Plants are generally low-growing and have multiple stems. Flowers closely resemble those of antelope horns (*A. asperula*), but the cup-shaped corollas of the flowers are much larger. Green antelopehorn is one of the main hosts for spring breeding monarchs in the south-central United States (Lynch and Martin 1993) and monarchs produced at southern latitudes play an important role in recolonizing the northern breeding range later in the season (Malcolm et al. 1993). Green antelopehorn is primarily a spring host plant, but sometimes re-emerges for a second growth cycle in response to prescribed burning during summer (Baum and Sharber 2012). Seed of this species is currently only available from a limited number of vendors.

**Bloom time:** May – August

**Flower color:** Corolla light green, corona purple tipped with white

**Maximum height:** 2 feet

**Estimated seeds per pound:** 68,500

## Imperiled Species

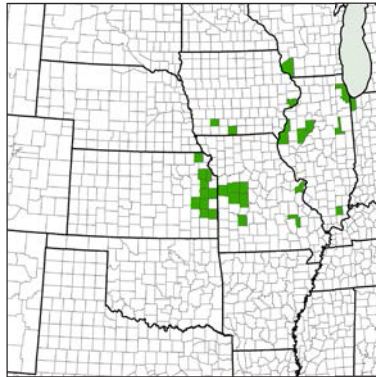
Mead's milkweed (*A. meadii*) has been federally listed as a threatened species since 1988. Four additional milkweeds that occur in the region are state listed as threatened or endangered. Where they are rare and declining, these species are in need of protection, habitat management, and targeted

restoration and recovery efforts. These milkweeds tend to have a narrower set of survival parameters than the more common species and may have more specific habitat requirements related to soil characteristics, moisture availability, and frequency of disturbance.

## *Asclepias meadii* Mead's milkweed



Photo: Retha Meier, Saint Louis University



USDA NRCS PLANTS  
in conjunction with the National Plant Data Team.

**Current distribution:** The species persists in small, scattered populations in Illinois, Iowa, Kansas, and Missouri, with nearly 91% of these populations located in 23 counties in eastern Kansas and west-central Missouri.

**Conservation status:** Federally threatened; endangered in Illinois, Iowa, and Missouri; S2 (imperiled) in Kansas.

**Flower color:** Corolla and corona green

**Bloom time:** May – June

**History:** Prior to European settlement, Mead's milkweed appears to have been widely distributed throughout the tallgrass prairie region, from Indiana to northeastern Kansas, and south into Missouri and Illinois.

Widespread fragmentation and destruction of tallgrass prairie resulted in the loss and decline of many Mead's milkweed populations.

**Reproductive Biology:** Mead's milkweed is a long-lived species with low reproductive rates and it may take up to 15 years for an individual plant to reach reproductive maturity (U. S. Fish and Wildlife Service 2003). The species is believed to have an outcrossing breeding system and does not usually produce seed when self-pollinated. Therefore, small populations with low genetic variability may have reduced reproductive capacity (U. S. Fish and Wildlife Service 2003).

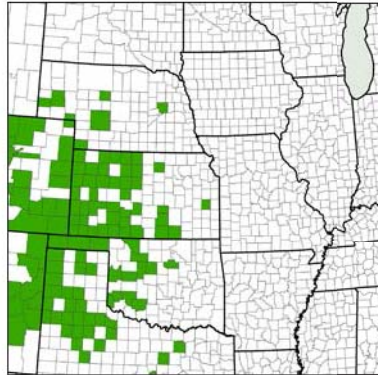
**Recovery efforts:** Since 1996, the USDA's Natural Resources Conservation Service, Kansas Biological Survey, and United States Fish & Wildlife Service have been working toward the restoration and recovery of Mead's milkweed. As part of this effort, the USDA-NRCS Plant Materials Center at Manhattan, Kansas has investigated the species' germination requirements and evaluated propagation and establishment techniques. Recent recovery efforts include the introduction of numerous juvenile plants to the Marais des Cygnes National Wildlife Refuge in Linn County, Kansas, during 2009. Monitoring of this introduction effort is ongoing and initial results have indicated a high survival rate.



## *Asclepias engelmanniana* Engelmann's milkweed



Photo: Leslie Landrum



Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Conservation status:** Endangered in Iowa.

**Flower color:** Corolla green, corona yellow

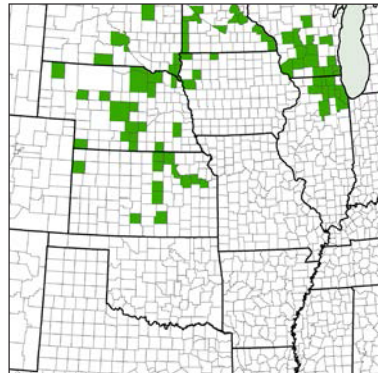
**Bloom time:** June – August

**Description:** This species' historical occurrences in western Iowa represent the northeastern extent of its documented range. Iowa is currently the only state in which Engelmann's milkweed has special conservation status. Across its range, the species grows in sandy or rocky limestone mixed and shortgrass prairies (Freeman and Schofield 1991) and also in swales, open sandy hillsides, draws, washes, and bottoms (Woodson 1954).

## *Asclepias lanuginosa* Sidecluster milkweed, woolly milkweed



Photo: Craig Freeman, R.L. McGregor Herbarium



Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Conservation status:** Endangered in Illinois; threatened in Iowa and Wisconsin; S1 (critically imperiled) in Kansas.

**Flower color:** Corolla pale yellow or green, corona cream to white

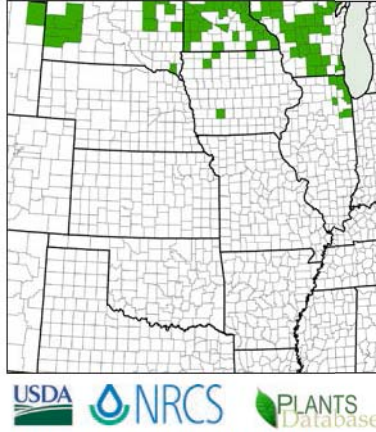
**Bloom time:** May – July

**Description:** Woolly milkweed grows in upland shortgrass and tallgrass prairies, including hillsides, in dry rocky, sandy, or gravelly soils. In Illinois, populations have been lost to overgrazing and gravel mining (Herkert and Ebinger 2002). In a study that compared the reproductive biology of 18 midwestern milkweeds, *A. lanuginosa* was one of two species that set the fewest seeds per stem (Betz and Lamp 1992). In Illinois, populations usually consist of only a few plants that rarely produce seeds (Herkert and Ebinger 2002). In addition to habitat loss, low reproductive output may be a factor in this species' decline.

## *Asclepias ovalifolia* Oval-leaf milkweed



Photo: Andy and Sally Wasowski, Lady Bird Johnson Wildflower Center



Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Conservation status:** Endangered in Illinois and Michigan; threatened in Wisconsin.

**Flower color:** Corolla and corona both cream to white, sometimes tinged with pink or yellow.

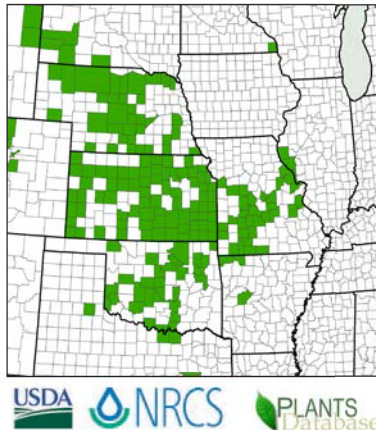
**Bloom time:** June – July

**Description:** The species' historical occurrences in northeastern Illinois represent the southeastern limit of its range. Oval-leaf milkweed prefers sandy or silt loam soils (Betz and Lamp 1992) and grows in prairies, savannas, open woods, and gravelly knolls (Woodson 1954; Herkert and Ebinger 2002).

## *Asclepias stenophylla* Slimleaf milkweed



Photo: Janice Lynn, Lady Bird Johnson Wildflower Center



Map: USDA-NRCS East Remote Sensing Laboratory in conjunction with the National Plant Data Team.

**Conservation status:** Endangered in Illinois, Iowa, and Minnesota; SH (possibly extirpated) in Arkansas; S1 (critically imperiled) in Louisiana.

**Flower color:** Corolla pale yellow or green, corona cream to white

**Bloom time:** June – August

**Description:** This species' occurrences in the three midwestern states where it is endangered represent the northeastern limit of its range. Slimleaf milkweed has a broad distribution across Nebraska, Kansas, and Oklahoma. It grows in sandy, rocky upland prairies, including hillsides and hay meadows. The transitioning of early successional habitats to forest is considered a threat to the species' survival in Illinois (Herkert and Ebinger 2002). In a study that compared the reproductive biology of 18 midwestern milkweeds, *A. stenophylla* was one of two species that set the fewest seeds per stem (Betz and Lamp 1992). In addition to habitat loss, low reproductive output may be a factor in this species' decline.



## Additional Species

Eight milkweeds have multiple documented occurrences within the region covered by this document, yet seed sources are scarce or certain habitat requirements limit their applicability in restoration efforts. While there is limited potential to include these species in conservation practices that involve seeding, their populations can be protected and managed where they occur.

*A. amplexicaulis* (clasping milkweed)  
*A. arenaria* (sand milkweed)  
*A. latifolia* (broadleaf milkweed)  
*A. pumila* (plains milkweed)

*A. purpurascens* (purple milkweed)  
*A. quadrifolia* (fourleaf milkweed)  
*A. variegata* (redring milkweed)  
*A. viridiflora* (green comet milkweed)

Finally, the following milkweeds have a very limited distribution within the central United States. For many of these species, their occurrences in the region represent the limit of their range.

*A. exaltata* (poke milkweed)  
*A. involucrata* (dwarf milkweed)  
*A. obovata* (pineland milkweed)  
*A. oenotheroides* (zizotes milkweed)

*A. perennis* (aquatic milkweed)  
*A. rubra* (red milkweed)  
*A. subverticillata* (horsetail milkweed)  
*A. uncialis* ssp. *uncialis* (wheel milkweed)

Please refer to the USDA PLANTS database (<http://plants.usda.gov>) for photos and range maps of these species.

## For More Information

The Xerces Society offers a range of resources to help with milkweed, monarch butterfly, and pollinator conservation:

- **Pollinator Conservation Program** ([www.xerces.org/pollinator-conservation](http://www.xerces.org/pollinator-conservation)). This provides nationwide training, consultation, and technical support on how to protect, manage, and restore habitat for bees, butterflies, and other beneficial insects.
- **Pollinator Conservation Resource Center**. An online database that includes pollinator plant recommendations, guidelines on establishing and protecting pollinator habitat, and lists of native plant nurseries for every region of the United States and Canada. <http://www.xerces.org/pollinator-resource-center/>
- **Guidelines for Creating Habitat**. In collaboration with the USDA-NRCS, Xerces has developed in-depth regional guidelines on installing and maintaining habitat for bees in the form of wildflower meadow or hedgerow plantings: <http://www.xerces.org/pollinator-conservation/agriculture/pollinator-habitat-installation-guides/>
- **Project Milkweed**. This initiative promotes milkweed conservation and increases native milkweed seed availability in key areas of the monarch butterfly's North American breeding range: [www.xerces.org/milkweed](http://www.xerces.org/milkweed).
- **Western Monarch Conservation Campaign**. A project focused on the conservation and management of western monarch overwintering habitat: <http://www.xerces.org/western-monarchs/>.

Other sources of information:

- **Monarch Watch**. Based at the University of Kansas, Monarch Watch has implemented the Monarch Waystation Program and the Bring Back the Monarchs campaign, and their website features numerous milkweed species profiles. Regionally appropriate milkweed transplants can be purchased from their online Milkweed Market. Please visit [www.monarchwatch.org](http://www.monarchwatch.org) to learn more about their work.
- **Farm Bill conservation programs**. For information on Farm Bill conservation programs that can support creation of pollinator habitat or plantings with milkweed on agricultural lands, please contact your local NRCS service center (<http://www.nrcs.usda.gov/wps/portal/nrcs/main/national/contact/local/>).

If you would like more information about using native milkweeds in restoration and revegetation efforts in the central United States, please contact:

Brianna Borders, Xerces Society Plant Ecologist (855-232 6639; [milkweed@xerces.org](mailto:milkweed@xerces.org)) or Chip Taylor, Director of Monarch Watch (888-824 4464; [chip@ku.edu](mailto:chip@ku.edu))

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Appendix to the Pollinator Plants of the Central United States: Native Milkweeds (*Asclepias* spp.)

**Milkweed Seed Vendors**

Following is a list of vendors that regularly have milkweed seed in stock. While some of them are not based in the central U.S. region that is described in the accompanying publication, they sometimes carry seed that is source-identified to the region. Before making a purchase, please ask vendors about the origin of their seed and endeavor to use seed that is sourced as locally to your property or project site as possible.

Companies denoted with an asterisk \* carry seed of at least one milkweed species in quantities of one pound or more. Companies not marked as such typically sell seed by the ounce or in small packets.

The companies are listed in alphabetical order. This list is not complete and does not constitute a guarantee of reliability or quality of product. The Natural Resources Conservation Service does not endorse any supplier and no discrimination is intended by omission.

**Allendan Seed Company**

1966 175th Lane, Winterset, IA 50273  
Ph: (515) 462-1241 Fax: (515) 462-4084  
[www.allendanseed.com](http://www.allendanseed.com)  
[allendan@allendanseed.com](mailto:allendan@allendanseed.com)

**Cardno JFNew Native Plant Nursery\***

128 Sunset Drive Walkerton, Indiana 46574  
Ph: (574) 586-2412 Fax: (574) 586-2718  
<http://www.cardnojfnew.com/Nursery.aspx>  
[nurserysales@cardno.com](mailto:nurserysales@cardno.com)

**Georgia Vines**

Ph: (912) 312-3762  
<http://www.georgiavines.com/>

**Hoksey Native Seeds**

12547 Hwy T-38S, Lynnville, IA 50153  
Ph: (641) 780-1539 Fax: (855) 594-3305  
[www.hokseynativeseeds.com](http://www.hokseynativeseeds.com)  
[info@hokseynativeseeds.com](mailto:info@hokseynativeseeds.com)

**Applewood Seed Company\***

5380 Vivian St., Arvada, CO 80002  
Ph: (303) 431-7333 Fax: (303) 467-7886  
[www.applewoodseed.com](http://www.applewoodseed.com)  
[sales@applewoodseed.com](mailto:sales@applewoodseed.com)

**Educational Science\***

P.O. Box 747, League City, TX 77574-0747  
Ph: (281) 554-9783 Fax: (281) 557-4340  
[www.educationalscience.com](http://www.educationalscience.com)  
[educationalscience@msn.com](mailto:educationalscience@msn.com)

**Hamilton Native Outpost, LLC**

16786 Brown Rd, Elk Creek, MO 65464  
Ph: (417) 967-2190 Fax: (417) 967-5934  
[www.hamiltonseed.com](http://www.hamiltonseed.com)  
[natives@hamiltonnativeoutpost.com](mailto:natives@hamiltonnativeoutpost.com)

**Ion Exchange, Inc.\***

1878 Old Mission Drive  
Harpers Ferry, IA 52146  
Ph: (563) 535-7231 Fax: (563) 535-7362  
[www.ionxchange.com](http://www.ionxchange.com)  
[hbright@ionexchange.com](mailto:hbright@ionexchange.com)



**Missouri Wildflowers Nursery**

9814 Pleasant Hill Road  
Jefferson City, MO 65109  
Ph: 573-496-3492 Fax 573-496-3003  
www.mowildflowers.net  
mowldflrs@socket.net

**Osenbaugh's Prairie Seed Farms\***

11009 542nd Street, Lucas, IA 505151  
Ph: (800) 585-2788 Fax (641) 766-6795  
www.prairieseedsfarms.com  
info@prairieseedsfarms.com

**Prairie Moon Nursery\***

32115 Prairie Lane, Winona, MN 55987  
Ph: (866) 417-8156 Fax: (507) 454-5238  
www.prairiemoon.com  
info@prairiemoon.com

**Shooting Star Nursery**

160 Soards Road, Georgetown, KY 40324  
Ph: (866) 405-7979  
www.shootingstarnursery.com

**Wilson Seed Farms, Inc.**

10872 1400 E Street, Tiskilwa, IL 61368  
Ph: (815) 878-8572 Fax: (815) 366-9050  
www.wilsonseed.com  
cwilson5@hotmail.com

**Native American Seed**

3791 N US Hwy 377, Junction, TX 76849  
Ph: (800) 728-4043 Fax: (800) 728-3943  
www.seedsource.com  
info@seedsource.com

**Pheasants Forever\***

1011 Alexander Avenue, Elba, NE 68835  
Ph: (308) 754-5339  
www.nebraskaPF.com  
pberthelsen@pheasantsforever.org

**Roundstone Native Seed, LLC\***

9764 Raider Hollow Road, Upton, KY  
42784  
Ph: (888) 531-2353 Fax: (270) 531-3036  
www.roundstoneseed.com  
sales@roundstoneseed.com

**Stock Seed Farms\***

Murdock, Nebraska  
Ph: (800) 759-1520  
www.stockseed.com

# Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies

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## Summary

1. Threats to migratory animals can occur at multiple periods of the annual cycle that are separated by thousands of kilometres and span international borders. Populations of the iconic monarch butterfly (*Danaus plexippus*) of eastern North America have declined over the last 21 years. Three hypotheses have been posed to explain the decline: habitat loss on the overwintering grounds in Mexico, habitat loss on the breeding grounds in the United States and Canada, and extreme weather events.

2. Our objectives were to assess population viability, determine which life stage, season and geographical region are contributing the most to population dynamics and test the three hypotheses that explain the observed population decline.

3. We developed a spatially structured, stochastic and density-dependent periodic projection matrix model that integrates patterns of migratory connectivity and demographic vital rates across the annual cycle. We used perturbation analysis to determine the sensitivity of population abundance to changes in vital rate among life stages, seasons and geographical regions. Next, we compared the singular effects of each threat to the full model where all factors operate concurrently. Finally, we generated predictions to assess the risk of host plant loss as a result of genetically modified crops on current and future monarch butterfly population size and extinction probability.

4. Our year-round population model predicted population declines of 14% and a quasi-extinction probability (<1000 individuals) >5% within a century. Monarch abundance was more than four times more sensitive to perturbations of vital rates on the breeding grounds than on the wintering grounds. Simulations that considered only forest loss or climate change in Mexico predicted higher population sizes compared to milkweed declines on the breeding grounds. Our model predictions also suggest that mitigating the negative effects of genetically modified crops results in higher population size and lower extinction risk.

5. Recent population declines stem from reduction in milkweed host plants in the United States that arise from increasing adoption of genetically modified crops and land-use change, not from climate change or degradation of forest habitats in Mexico. Therefore, reducing the negative effects of host plant loss on the breeding grounds is the top conservation priority to slow or halt future population declines of monarch butterflies in North America.

**Key-words:** agricultural intensification, annual cycle, conservation planning, genetically modified organisms, matrix modelling, migratory connectivity, transboundary conservation

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## Introduction

Hemispheric migrations of wildlife involving billions of individuals each year are in widespread decline (Robbins *et al.* 1989; Bolger *et al.* 2008; Brower *et al.* 2012). Migratory animals face multiple threats at different portions of the annual cycle that are often separated by vast geographical distances (Webster *et al.* 2002), which pose enormous challenges for predicting population abundance and designing effective management plans (Martin *et al.* 2007; Norris & Marra 2007; Small-Lorenz *et al.* 2013). Underscoring good management is an understanding of how various environmental and anthropogenic threats interact to influence population dynamics, through their impact on vital rates, in the face of global change. Addressing threats to population viability of migratory animals therefore requires integrating detailed information of how individuals move, survive and reproduce throughout the annual cycle and respond to these threats (Webster *et al.* 2002; Norris & Marra 2007; Taylor & Norris 2010; Jenouvrier 2013).

Monarch butterflies (*Danaus plexippus*), which undergo a long-distance migration between breeding and non-breeding locations typical of vertebrates, have traditionally been considered most vulnerable to disturbance on the overwintering grounds. In Mexico, forest habitat loss (Brower *et al.* 2002) and severe weather patterns (Oberhauser & Peterson 2003; Brower *et al.* 2004) are known to affect local butterfly population abundance by increasing the probability of catastrophic mass mortality events (Anderson & Brower 1996; Brower *et al.* 2012). Alternatively, declines of monarch butterflies may also be attributed to habitat loss that could occur at multiple locations and time periods of the breeding cycle. Reduction in host plants (various milkweed species, *Asclepias*) due to land-use change (mostly urbanization) and agricultural practices, such as the adoption of genetically modified, herbicide-resistant corn and soybean crops, that lower density of host plants in agricultural fields on the breeding grounds (Oberhauser *et al.* 2001; Brower *et al.* 2012; Pleasants & Oberhauser 2013) is predicted to increase competition for food among larvae leading to decreases in immature survival (Flockhart, Martin & Norris 2012).

Given that the conservation of monarch butterflies, like many migratory species, is a responsibility shared by multiple countries (Commission for Environmental Cooperation 2008), a quantitative assessment of year-round population dynamics is critical for guiding effective trans-boundary conservation planning and assessing risk of extinction in the wild. Our objectives were to (i) assess the long-term viability trend and cumulative quasi-extinction probability (<1000 individuals) for monarch butterflies over the next 100 years given projected land-use changes that modify host plant abundance across the breeding grounds and concurrent future climate trends and deforestation rates that alter the frequency of winter mass

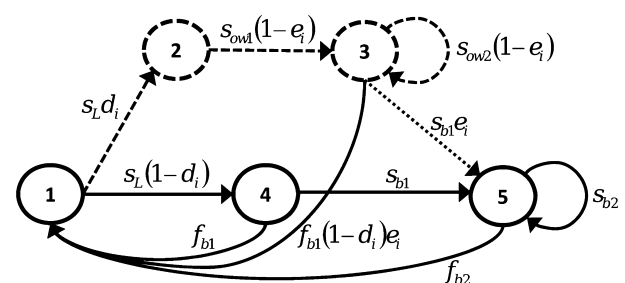
mortality events on the wintering grounds; (ii) use transient elasticity analysis (the relative sensitivity values which sum to 1) of the projected population to determine which life stage, season and geographical region across the annual cycle are contributing the most to explain population declines of migratory monarch butterflies; (iii) test the three hypotheses of population decline by comparing the singular effects of habitat loss on the breeding ground, habitat loss on the wintering grounds and climate change to a full model where all factors operate concurrently; and (iv) explore the effects of host plant loss on the breeding ground as a result of adoption of genetically modified crops on future monarch butterfly population size and the risk of extinction.

## Materials and methods

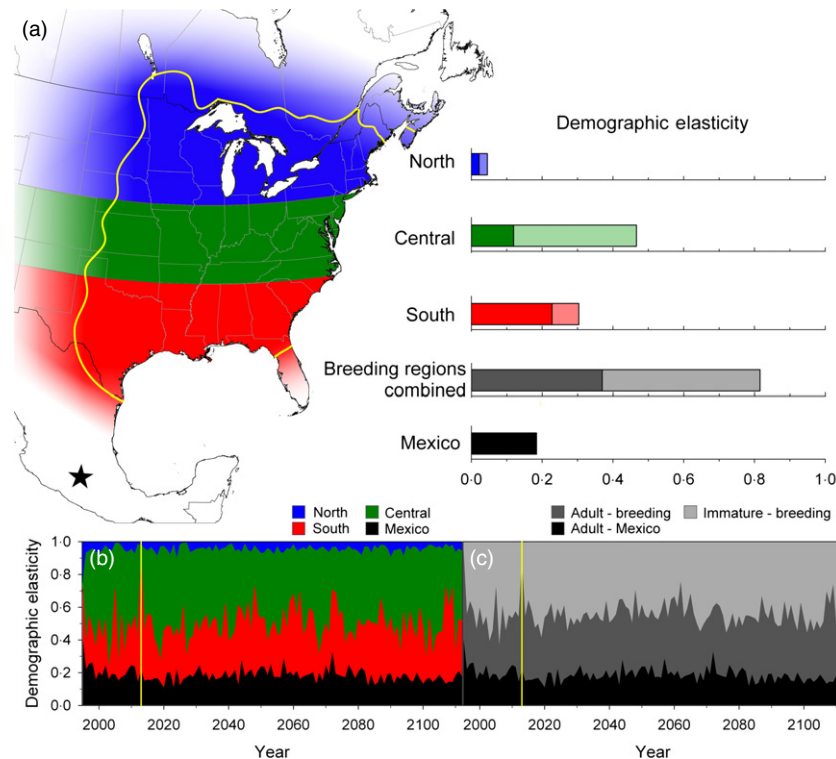
Our population model required parameter estimates of survival, fecundity and migration throughout the annual cycle (Fig. 1). We considered one overwintering and three breeding regions (Fig. 2a) to parameterize a spatially structured, two-cohort, stochastic and density-dependent periodic projection matrix model (Hunter & Caswell 2005) for monarch butterflies. The model structure (Fig. 1) used a two-cohort approach to differentiate butterflies in diapause that migrate to Mexico from reproductively active butterflies because these cohorts have different physiological and demographic processes (Brower 1995). We therefore had five life stages: an immature stage that included all developmental transitions from egg to eclosion and then first- and second-month or greater (hereafter second month) vital rate estimates for each cohort of adults that captures differences in survival and reproduction (Fig. 1).

### POPULATION MODEL

The model took the form  $\vec{n}(t+1) = A_t \vec{n}(t)$  where the global transition matrix  $A$  at a given month  $t$  is used to project the population vector, that is, arranged as the spatial distribution of each



**Fig. 1.** The life cycle graph of monarch butterflies, characterized by five stages. The immature (1) stage includes egg, larval and pupal development until eclosion. Eclosed butterflies in their first (2) or second or more (3) month of life are in a reproductive diapause (dashed lines). Only eclosed butterflies in their first (4) or second or more (5) month of life in breeding condition (solid lines) produce offspring. The dotted line between (3) and (5) represents overwintered butterflies that emerge from diapause in April and become reproductively active in the South. Descriptions of the variables are provided in Table S1 (Supporting information).



**Fig. 2.** The geographical regions occupied by monarch butterflies throughout the annual cycle in eastern North America and their associated long-term demographic elasticities between regions, seasons and life stages. (a) Butterflies overwinter in Mexico (black star) and breed in the South (red), Central (green) and North (blue) regions. The yellow outline indicates the monarch breeding habitat study area that was used to quantify milkweed abundance (See Supporting Information). Population abundance was most sensitive to vital rates in the Central region, followed by the South and Mexico, and least sensitive to vital rates in the North. The Central region was most sensitive to perturbation of immature vital rates (light green) compared to adults (dark green), whereas the opposite pattern was found in the South. Population abundance was more sensitive to vital rates on the breeding grounds (South, Central and North regions combined) than the non-breeding grounds (Mexico). On the breeding grounds, sensitivity was almost equal between adult (dark grey) and immature (light grey) life stages. (b) Annual trends of the demographic elasticity of monarch population abundance to perturbation of vital rates among regions and (c) between the breeding and non-breeding portions of the annual cycle. The annual demographic elasticity values vary annually owing to stochastic population processes; however, the sensitivity patterns to the left (historic) and right (future) of the vertical yellow line remain relatively consistent.

stage (Hunter & Caswell 2005), from  $n(t)$  to  $n(t + 1)$ . Within each time step  $t$  of 1 month,  $A_t$  included both migration among and demography within the four geographical regions  $i$  of the five life stages using the block-diagonal formulation and vec-permutation approach (Hunter & Caswell 2005). In this arrangement, butterflies first move between regions before demographic events like reproduction occur, in order to reflect that females are reproductively active during the rapid re-colonization of eastern North America over successive generations (Malcolm, Cockrell & Brower 1993; Flockhart *et al.* 2013). The process is repeated for each of 12 months to project the population over an annual cycle.

The block-diagonal matrix organizes the demographic processes and transitions among life stages within regions (Hunter & Caswell 2005). The demographic vital rates represent survival of immature ( $s_L^i$ ), overwinter survival for butterflies in their first and second month of diapause ( $s_{ow1}^i$ ,  $s_{ow2}^i$ ), survival of adults in their first and second month of breeding ( $s_{b1}^i$ ,  $s_{b2}^i$ ), and fecundity of breeding butterflies in their first ( $f_{b1}^i$ ) and second ( $f_{b2}^i$ ) months (Fig. 1). The terms  $d_i$  and  $e_i$  permit the transition of generations between reproductive to diapause life-history stages in autumn (which occurs during immature development; Goehring &

Oberhauser 2002) and the emergence from diapause to breeding condition at the end of the winter, respectively. The migration process was structured with the block-diagonal dispersal matrix that accounts for migration of adults between regions. For each adult cohort, migration included both the transition rate among regions ( $t_{ij}$ ) and survival during migration between these same regions ( $s_{ij}$ ). The Supporting Information provides details of the model structure.

#### VITAL RATE ESTIMATION

The population response to the effects of habitat loss and climate change, key environmental factors that are thought to strongly influence population size of monarch butterflies (Brower *et al.* 2012), occurs through changes to vital rates. We present the results of models to estimate milkweed abundance on the breeding grounds and weather-induced mass mortality events in Mexico that were incorporated into estimates of larval survival and adult winter survival probability, respectively. Detailed analysis of each vital rate estimate, as well as the milkweed abundance and weather-induced mass mortality models, is available in the Supporting Information.



### Fecundity

We estimated fecundity of first-month and second-month adults (Table S1, Supporting information:  $f_{b1}^i, f_{b2}^i$ ) using estimated lifetime egg output reported in Oberhauser (1997). We assumed that females laid 75% of their total eggs in the first month and 25% in the second month. Sex ratio of offspring was assumed to be 50:50.

### Migration

Migration combines information on migratory connectivity (Webster *et al.* 2002; Norris & Marra 2007) and survival during migration (see below) to estimate the transition probability of adults flying between different regions at each time step. Following the two-cohort structure of the model, we differentiate rates between non-reproductive butterflies that are on fall migration to Mexico and reproductively active butterflies that can move between breeding regions.

The timing of migration of non-breeding butterflies to Mexico follows a relatively predictable pattern by latitude (Taylor 2013). We incorporated these temporal migration patterns in our model by assuming that butterflies depart to Mexico from the north during September, from the central during October and from the south during November. Collectively these butterflies arrived at the overwintering colonies in December where they remained until April when they became reproductively active (Brower 1995).

Reproductive monarch butterflies colonize the breeding grounds over successive generations (Malcolm, Cockrell & Brower 1993; Miller *et al.* 2011, Flockhart *et al.* 2013). We assumed the main cohort of butterflies colonized the south in April, the central in May and the north in June (Cockrell, Malcolm & Brower 1993) and the last breeding generation would occur in August in the north, September in the central and October in the south (Brower 1995; Calvert 1999; Baum & Sharber 2012; Flockhart *et al.* 2013).

We calculated migration rates ( $t_{ij}^i$ ) of breeding butterflies based on published information in Flockhart *et al.* (2013) who used stable-hydrogen and stable-carbon isotopes to assign a geographical origin of captured butterflies. Using the assigned geographical region as the origin and the capture region as the destination, we cross-tabulated origin and destination regions to produce a contingency table of relative frequency by dividing the number assigned to each origin region by the marginal total of the destination regions. Using this approach, we calculated the migration between the four regions (origin included Mexico for butterflies that overwintered; see Supporting Information) for each month during the year.

### Breeding-season survival

First- and second-month adult female survival (Table S1, Supporting information, Fig. 1:  $s_{b1}^i, s_{b2}^i$ ) estimates came from longevity measures of captured wild female butterflies (Herman & Tatar 2001). Immature survival ( $s_j^i$ ) was the cumulative survival from egg to eclosion as an adult butterfly and considered the product of a density-dependent survival relationship based on larval competition for milkweed host plants (Flockhart, Martin & Norris 2012), density-independent larval survival (Oberhauser *et al.* 2001) and pupal survival (Oberhauser 2012).

We applied the findings of Flockhart, Martin and Norris (2012) who showed larval survival probability declined as the average number of eggs per milkweed stem increased. Therefore, calculating the density-dependent response required an estimate of milkweed abundance in each region. To estimate the total number of milkweed stems, we multiplied the land area of different land-cover types (e.g. Taylor & Shields 2000) by the proportion of infested area for each land-cover type (e.g. Hartzler & Buhler 2000; Hartzler 2010; Pleasants & Oberhauser 2013) and the number of milkweed stems within infested areas (see Supporting Information). To understand milkweed abundance change over time, we estimated annual rates of land-cover conversion using data from 1982 to 2007 (U.S. Department of Agriculture 2009) and used nonlinear models to estimate the expected changes in adoption of genetically modified, herbicide-resistant corn and soybean crops (Hartzler 2010; Pleasants & Oberhauser 2013). Details of land-cover change and associated dynamic milkweed abundance are presented in the Supporting Information.

We calculated density-independent larval survival from egg to pupation using estimates from Oberhauser *et al.* (2001) who presented counts of 5th instar larvae relative to counts of eggs in non-agricultural areas, agricultural fields and field margins in four geographical regions that spanned the breeding range. Tachinid flies parasitize monarch larvae that results in mortality realized during the pupa stage, so pupal survival was assumed as one minus the marginal parasitism rate of fifth instars based on 11 years of data following Oberhauser (2012). Mortality during the pupal stage was therefore assumed to result solely from tachinid fly parasitism and provides a suitable way to incorporate this important source of immature mortality on monarch population dynamics (Oberhauser *et al.* 2007).

### Migration survival

Evidence for Lepidoptera suggests mortality during migration to be low relative to the stationary portions of the annual cycle (Chapman *et al.* 2012; Stefanescu *et al.* 2013), whereas the opposite pattern has been found for vertebrates (Muir *et al.* 2001; Sillett & Holmes 2002). Few data exist to estimate these mortality rates directly and there is currently no published information for monarch butterflies. In the absence of empirical estimates, the opinions of experts can provide valuable information to understand population processes (Martin *et al.* 2012a).

We used an expert elicitation exercise to estimate the survival of monarch butterflies during both spring and fall migration ( $s_{ij}^i$ ; Table S1, Supporting information). The exercise consisted of independent elicitation of survival estimates, an anonymous review of the group results, and a second round of elicitations where experts were allowed to modify their original responses after having seen the group results (Martin *et al.* 2012a). Each expert provides a worst-case, average-case and best-case estimate of the probability of survival for (i) butterflies migrating to the overwintering colonies during autumn migration, (ii) overwintered adult monarch butterflies that migrate from Mexico to the south and (iii) first- or second-generation breeding butterflies born in the south that re-colonize the rest of the breeding distribution. We calculated the mean and standard deviation for the average-case values between each pairs of regions provided by experts and found that the variation of survival implemented into the matrix model contained both the mean worst-case and best-case estimates provided by experts, suggesting that the estimates

of survival during migration generated during simulations of the model captured a range of expected survival rates (see Supporting Information).

As survival during migration was the only vital rate used in the population model not based on empirical data, we present a sensitivity analysis in the Supporting Information of how well the worst-case, average-case and best-case survival estimates predicted the observed monarch butterfly population decline. The results suggest that both the average-case and best-case scenario estimates better reflect monarch butterfly population dynamics but that the predictions from models that incorporate the average-case scenario more closely reflect the observed population decline (see Supporting Information).

### Overwintering survival

The probability of survival for overwintering adult butterflies ( $s_{ow1}^i, s_{ow2}^i$ ) was a product of a baseline survival in the presence of predators (Brower & Calvert 1985; Glendinning, Alonso Mejia & Brower 1988) and catastrophic mortality events caused by extreme weather phenomena (Brower *et al.* 2004). Birds were estimated to kill about 9% of all butterflies in colonies (Brower & Calvert 1985), whereas mice are predicted to kill about 4% of the population (Glendinning, Alonso Mejia & Brower 1988). To estimate mortality, we divided the estimated number of depredated butterflies from Brower *et al.* (1985) and Glendinning, Alonso Mejia and Brower (1988) by butterfly densities (butterflies/ha) from the Jolly-Seber estimates in Calvert (2004) to correct for potentially biased estimates of population density in the wintering colonies. Assuming that predation by birds and mice is independent, multiplying the product of the two survival estimates yielded the baseline overwinter survival (see Supporting Information).

Stochastic mass mortality events in the overwintering colonies can kill significant numbers of the entire eastern population during a single storm (Brower *et al.* 2004). The magnitude of each mortality event is the interplay between ambient temperature, precipitation and exposure that determine body temperature, and hence freezing risk, of monarch butterflies (Anderson & Brower 1996). We used a logistic function to model the proportion of the total overwintering population that would die from extreme weather. The model included the effects of temperature, precipitation and changes in exposure (see Supporting Information). The addition of an exposure parameter incorporates the 'blanket effects' (Anderson & Brower 1996) offered by high-quality forest habitat that was assumed to be lost at 1.3% per year (Brower *et al.* 2002; Ramírez, Azcárate & Luna 2003; López-García & Alcántara-Ayala 2012; Vidal, López-García & Rendón-Salinas 2014).

Temperatures and rainfall patterns are predicted to change over the next 100 years in Mexico (Sáenz-Romero *et al.* 2010) and these changes are predicted to influence monarch mass mortality events (Oberhauser & Peterson 2003). Using the location and elevation of the monarch colonies (García-Serrano, Lobato Reyes & Mora Alvarez 2004), we extracted monthly (December to March) current and future temperature under the A2 scenario of the CGCM3 (T62 resolution) climate model that assumes high greenhouse gas emissions and a growing human population, presented in Sáenz-Romero *et al.* (2010). For each month, we fit a linear regression of predicted mean minimum temperature using data from the years 2000 (current), 2030, 2060 and 2090 as our predicted climate projection in the overwintering colonies. Variation in daily temperatures was assumed to remain consistent

over time and was estimated from daily minimum temperatures from five federal weather stations representative of the overwintering colonies (see Supporting Information). We assumed daily rain events >10 mm would result in butterflies being wetted and making them more vulnerable to freezing risk (Anderson & Brower 1996) and assumed that the probability of a rain event of >10 mm between December and March summarized from the weather station data would remain consistent. The matrix population model randomly selected a daily probability of a large rain event and a minimum temperature to calculate the daily survival between December 1 and March 30. The product of these daily mortality estimates represented the population-level stochastic mortality rate of each year of the model (see Supporting Information).

### ANALYSIS

We initiated the population model using the population size observed in 1994 (Rendón-Salinas & Tavera-Alonso 2014) to assess the model fit from the first 19 years of the simulation (1995–2013) and then projected the population for 100 years and calculated the stochastic population growth rate ( $\log \lambda_s$ ) and 95% confidence interval from 1000 simulations. Model fit was assessed by testing the standard deviates of the population growth rates from observed and projected population sizes (McCarthy *et al.* 2001). The cumulative probability of quasi-extinction was determined using a binomial model that regressed the counts of the number of simulations that had gone extinct by a given year. To test between the three hypotheses, we divided the mean population size from a simulation with each effect by the population size of the full model and used linear models to regress differences in population size against year. A slope different from zero indicates that threat alone would cause a larger (in the case of positive slope) future population than the full model that considers all threats simultaneously.

To understand the factors that limit population size of monarch butterflies, we estimated monthly transient elasticities (the relative sensitivity values which sum to 1) of the total species abundance to perturbation of the migration and demographic vital rates (Caswell 2007). To make general predictions of the sensitivity of population growth to changes in vital rates throughout the annual cycle, we summed the demographic transition elasticity values across life stages (immature, adult), life-history events (breeding, non-breeding) or regions (Mexico, South, Central, North). We ran all simulations using Matlab R2009.

## Results

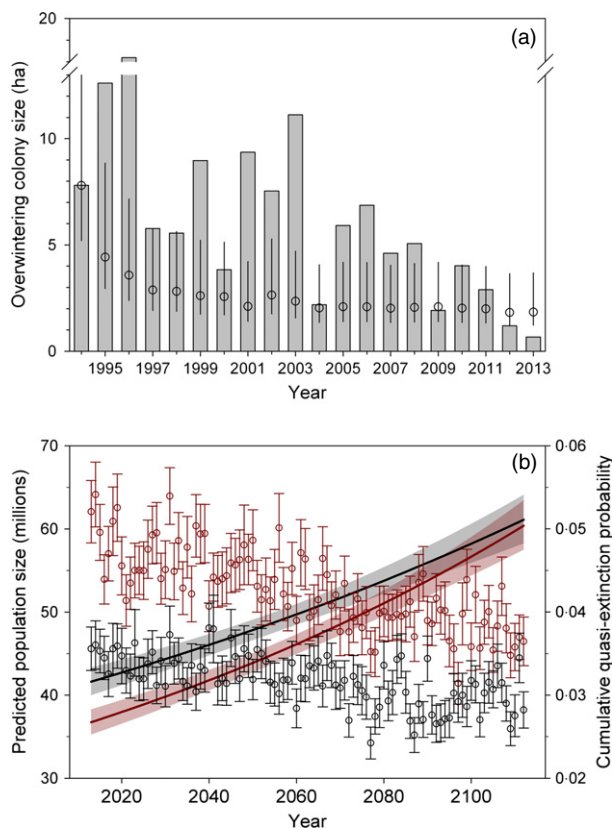
### POPULATION TREND AND EXTINCTION PROBABILITY

Population size estimates from our model were not significantly different from the observed data ( $t = -0.4889$ ,  $P = 0.63$ ; Fig. 3a) and predict that, if land-use and climate change continue as expected, population size will decline by an additional 14% within the next 100 years (Fig. 3b). Furthermore, under these conditions, we predict that the cumulative probability of quasi-extinction of <1000 butterflies remaining in the population over 100 years is >5% (Fig. 3b). Overall, the stochastic population growth rate was predicted to be  $-0.0332$  (95% CI:  $[-0.4028, 0.3364]$ )

(Fig. 3b) which is consistent with the population growth rate observed over the past two decades ( $r = -0.048$ , 95% CI:  $[-0.186, 0.089]$ ; Brower *et al.* 2012; Rendón-Salinas & Tavera-Alonso 2014).

#### SENSITIVITY OF POPULATION ABUNDANCE

Population abundance was more sensitive to land-use and climate changes on the breeding grounds (mean  $0.816 \pm 0.004$  SEM) than on the wintering grounds



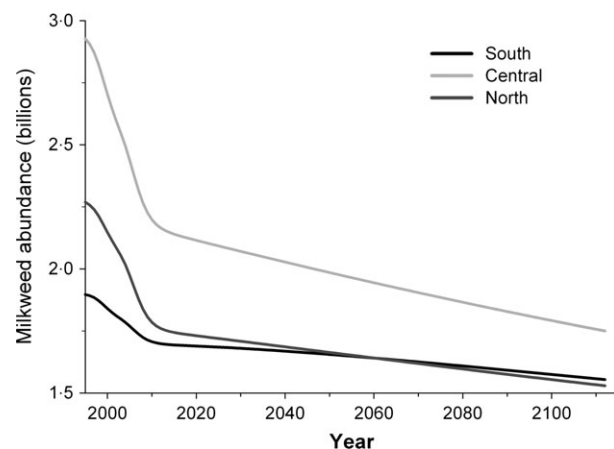
**Fig. 3.** Monarch butterfly population size is projected to decrease with a corresponding increase in quasi-extinction probability. (a) The model-derived mean predicted overwintering colony size (points) fit the observed monarch butterfly overwintering colony size (bars; Brower *et al.* 2012; Rendón-Salinas & Tavera-Alonso 2014). Overwintering population size is the area (in hectares) occupied by clustering monarch butterflies. For predicted colony size, the points represent the mean density (50 million ha<sup>-1</sup>), while the upper (25 million ha<sup>-1</sup>) and lower (75 million ha<sup>-1</sup>) error bars represent the observed lower and upper population density estimate (Brower *et al.* 2004) assuming a 1 : 1 sex ratio. (b) Projected mean population size ( $\pm$ SE, circles and error bars) and probability ( $\pm$ 95% CI, line and shading) of quasi-extinction (<1000 individuals) in eastern North America in December of each year from 2013 to 2112. The full model (black) which includes the effects of genetically modified crops is compared to a simulation with no genetically modified crops (red) and shows the presence of genetically modified crops predict monarch butterfly populations being lower with a higher probability of quasi-extinction over the next half-century. The population values represent the number of females since the model only considered female butterflies.

( $0.184 \pm 0.004$ ; Fig. 2a). Decomposition of these sensitivities showed that larvae ( $0.446 \pm 0.007$ ) were more sensitive compared to adults ( $0.370 \pm 0.007$ ) on the breeding grounds (Fig. 2a). At a regional scale, the total butterfly abundance was more sensitive to land-use and climate change impacts on the vital rates within the Central breeding region ( $0.446 \pm 0.010$ ) than within the South breeding region ( $0.304 \pm 0.010$ ) or Mexico ( $0.184 \pm 0.004$ ), whereas butterfly abundance was least sensitive to impacts in the North breeding region ( $0.045 \pm 0.002$ ; Fig. 2a). Further decomposition between life stages and regions suggests that the patterns in the Central region resulted from sensitivity of perturbation of immature vital rates rather than adults (Fig. 2a). In contrast, in the South, butterfly abundance was more sensitive to disturbance of adult vital rates compared to vital rates of the immature stage (Fig. 2a).

Although annual elasticities varied between years, the historic and future sensitivity patterns were predicted to remain relatively consistent over time. For example, population abundance was about four times more sensitive to changes in vital rates on the breeding grounds than wintering grounds throughout the study (Fig. 2b) despite a reduced probability of mass mortality events in Mexico over time (Fig. S1, Supporting information). Furthermore, changes in butterfly abundance were about 1.3 times more sensitive to changes in vital rates of adults than those of larvae both at the start and end of the study (Fig. 2c) despite a reduction in milkweed abundance across the breeding distribution (Fig. 4).

#### THREATS TO POPULATION VIABILITY

Under current conditions, the annual probability of a mass mortality event on the wintering grounds was about

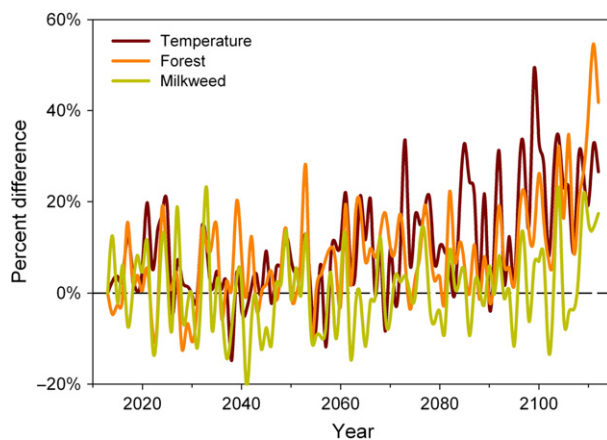


**Fig. 4.** Predicted changes of milkweed abundance in each breeding region between 1995 and 2112. In each region, milkweed was estimated by multiplying milkweed density by the area of different land-cover types infested with milkweed. Changes in milkweed abundance reflect predicted adoption rates of genetically modified, herbicide-tolerant corn and soybean crops and annual land-use changes.



11% and a reduction in forest cover increased the probability of these events (Fig. S1, Supporting information). Surprisingly, under projected climate change, the chance of butterflies being killed due to severe weather was predicted to decline. For example, winter mortality probabilities of adults under current conditions (11% per year) were eight times higher compared to 2030 (1%), 73 times higher compared to 2060 (<0.2%) and 665 times higher compared to 2090 (<0.02%; Fig. S1, Supporting information). However, over time, the decreasing probability of a mass mortality event caused by rising temperatures trumped any negative effects caused by reduction in forest cover (Fig. S1, Supporting information). Simulations of population dynamics that considered only the effects of forest loss or climate change in Mexico predicted higher population sizes compared to the full model that considered all effects simultaneously (Fig. 5).

Between 1995 and 2013, our model estimated that 1.49 billion individual milkweed stems were lost, representing a 21% decline in milkweed abundance (Fig. 4). Over the past two decades, the Central region, which was the most sensitive to perturbation of vital rates, had the most rapid loss of milkweed (Fig. 4) which resulted from recent widespread adoption of genetically modified, herbicide-resistant corn and soybean crops associated with industrial agriculture. Currently, we estimated there were more than 5.6 billion



**Fig. 5.** Reductions in milkweed host plants drive monarch butterfly population decline. The proportional difference in projected mean population size of monarch butterflies over time under the effects of milkweed loss (yellow), forest loss (orange) or changes in temperature (red) relative to the full model that includes all three effects (dashed line). Linear models that regressed population size against year were significant for temperature ( $\beta = 0.0023$ ,  $P < 0.001$ ) and forest ( $\beta = 0.0021$ ,  $P < 0.001$ ) but not for milkweed ( $\beta = 0.0005$ ,  $P = 0.09$ ), indicating no difference in projected population sizes of the full model compared to one that only considered milkweed and hence milkweed is the driving factor of monarch population decline. The models for each effect controls for the others; for example, the milkweed model includes the effects of declines of milkweed host plant abundance on the breeding grounds while controlling for the effects of climate change (no change in temperatures over time) and deforestation (no deforestation over time) in Mexico.

milkweed stems in the study area with the majority (67%; 3.7 billion stems) occurring in agriculture-intensive landscapes (Table 1). Land held in the public trust for the maintenance of biodiversity (e.g. Conservation Reserve Program lands, road right-of-ways) contained 18% of all milkweed plants. Importantly, road right-of-ways accounted for almost 548 million plants (10% of all milkweeds; Table 1). Our model predicts the rate of milkweed decline to slow into the future with milkweed abundance being 14% lower than current conditions, although this still represents a loss of 770 million milkweed plants over the next 100 years (Fig. 4). Overall, genetically modified, herbicide-resistant crops have increased the current, and predicted future, extinction probability of monarch butterflies in eastern North America (Fig. 3b).

## Discussion

Our results suggest both climate change and deforestation had less influence on projected population declines compared to the effects of milkweed declines on the breeding grounds. These results are contrary to the long-held belief that monarch butterflies were most vulnerable to disturbance on the wintering grounds since they congregate in a small area at high densities (Brower *et al.* 2002, 2004). Indeed, this was some of the motivation for multiple Mexican presidential decrees that protected butterfly overwintering habitats (Commission for Environmental Cooperation 2008) and recent successful efforts to curb illegal deforestation activities (Vidal, López-García & Rendón-Salinas 2014). Despite a reduced probability of catastrophic mortality events on the wintering grounds, sensitivity to this life-history stage compared to the breeding season remained relatively fixed because mortality is infrequent, stochastic and density-independent (Brower *et al.* 2004). In other words, even if monarchs adjust their behaviour to deal with changing habitat availability (Sáenz-Romero *et al.* 2012) or experience different future temperature and precipitation regimes (Oberhauser & Peterson 2003; Sáenz-Romero *et al.* 2010), population viability is expected to remain less sensitive to mortality on the wintering grounds than to changes in demographic rates on the breeding grounds.

Overall, observed monarch butterfly population decline and future increased extinction risk are largely driven by conditions on the breeding grounds, particularly in the Corn Belt region of the United States (Oberhauser *et al.* 2001; Brower *et al.* 2012; Pleasants & Oberhauser 2013). Given the demographic importance of the Central region and its direct link to overwintering population size in Mexico (Wassenaar & Hobson 1998; Flockhart *et al.* 2013), the rapid loss of milkweed projected for this region attributable to land-cover changes and shifts in agricultural practices is a large concern (Hartzler 2010; Brower *et al.* 2012; Pleasants & Oberhauser 2013). As monarchs are larval host plant specialists, changes in milkweed abundance directly influence vital rates, first through



**Table 1.** The proportion of milkweed stems in eastern North America in 2013. Estimates are among breeding regions, landscape protection classification and land-cover types. The total number of estimated milkweed plants was 5 604 106 046

	South		Central		North	
Unprotected	0.84		0.84		0.76	
Crop <sup>a</sup>		0.698		0.880		0.862
Pasture		0.117		0.083		0.108
Rangeland		0.046		0.016		0.024
Wetland		0.139		0.020		0.006
Protected	0.16		0.16		0.24	
Crop <sup>b</sup>		0.177		0.282		0.544
Pasture		0.007		0.009		0.012
Rangeland		0.005		0.004		0.005
Wetland		0.224		0.108		0.006
Right-of-ways		0.587		0.597		0.433
Percentage of total	0.303		0.384		0.313	
Total milkweed	1 696 459 725		2 154 696 122		1 752 950 199	

<sup>a</sup>Includes the effects of genetically modified corn and soybean crops on milkweed abundance, see text for details.

<sup>b</sup>Cropland assumed to have milkweed density of Conservation Reserve Program lands.

intraspecific larval competition (Flockhart, Martin & Norris 2012) or alternatively, by preventing females from laying a full egg complement (Zalucki & Lammers 2010), although the latter has never been empirically demonstrated. Ultimately, understanding the mechanism by which milkweed reduction limits population abundance will have important implications towards conservation planning.

Our results imply that conserving monarch butterflies by addressing the negative impacts of changing land-use and the adoption of genetically modified, herbicide-resistant crops on host plant abundance is the highest conservation priority. These conclusions should not be misconstrued as implying that efforts towards improving the social, economic and environmental conditions on the wintering grounds are not important, but rather, that inaction in one location during a portion of the annual cycle can undermine conservation efforts in other portions of the annual cycle (Myers *et al.* 1987; Martin *et al.* 2007; Sheehy *et al.* 2010; Vidal, López-García & Rendón-Salinas 2014). Specifically, increasing host plant abundance in the South and Central regions of the United States is expected to translate into the largest benefit to species viability. While planting milkweeds in gardens of private citizens and publicly held lands such as road right-of-ways may be the easiest locations to focus immediate conservation efforts given the limited supply of milkweed seeds, overall, these efforts may be insufficient to negate the ongoing annual loss of milkweed plants let alone address the massive habitat losses observed over the last two decades due to industrial agricultural practices (Hartzler 2010; Pleasants & Oberhauser 2013). Furthermore, the spatial arrangement of milkweed may influence population response (e.g. Zalucki & Lammers 2010) highlighting that both recovery efforts and threats are dynamic and spatially explicit (Brower *et al.* 2002, 2012; Commission for Environmental Cooperation 2008); thus, stochastic population dynamics should be incorporated into cost-effective conservation planning options

(Baxter *et al.* 2006; Martin *et al.* 2007; Pichancourt *et al.* 2012) to aid monarch butterfly population recovery.

Limited data will affect what we can infer about how ecological relationships interact to influence population dynamics across space and time. In extreme cases, no data exist to estimate vital rates. For example, there are virtually no empirical estimates of survival during migration for any terrestrial migratory animal (for rare exceptions see: Ward *et al.* 1997; Sillett & Holmes 2002; Chapman *et al.* 2012). In such cases, we may be limited to using educated guesses or surveys of experts as to the likely range of empirical values (Martin *et al.* 2012a). Drawing inference from model results based on sparse data must therefore be done cautiously, particularly when the sensitivity of data-limited vital rates is high, but is often necessary when investigating steep population declines of threatened species and where conservation success depends on timely decision-making (Martin *et al.* 2012b). For monarch butterflies, subsequent sensitivity analysis suggests that the values elicited from butterfly experts were robust to observed population dynamics and that true survival during migration is expected to be equal or slightly higher than what was provided by experts.

Population declines among migratory species have generated hypotheses that populations are limited by conditions on the breeding grounds (Robinson *et al.* 1995), the non-breeding grounds (Robbins *et al.* 1989; Sherry & Holmes 1996), during migration (Bolger *et al.* 2008) or a combination of these factors (Kareiva, Marvier & McClure 2000; Brower *et al.* 2012). However, quantifying which environmental and anthropogenic factors drive population dynamics at global extents is a complex issue because it depends on how we integrate migratory connectivity and population processes across the annual cycle (Sherry & Holmes 1996; Kareiva, Marvier & McClure 2000; Faaborg *et al.* 2010) and the quality of the data available.

Overall, the general modelling approach we promote could be applied to any migratory species because it

incorporates recent methods to delineate migratory connectivity (Webster *et al.* 2002), how seasonal interactions influence vital rates via density dependence (Norris & Marra 2007), and established approaches of evaluating spatial population dynamics across the annual cycle (Hunter & Caswell 2005; Caswell 2007). Ultimately, the ability to quantify contributions to population growth rate across the annual cycle provides a tractable way to measure the robustness of international conservation programmes (Bull *et al.* 2013) and has important legal implications for conserving threatened wildlife that migrate between countries that classify and protect species-at-risk differently (Fischman & Hyman 2010).

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## Author contributions

D.T.T.F., D.R.N. and T.G.M. designed the research. D.T.T.F. analysed the data. J.-B.P. wrote the code of model. D.T.T.F., D.R.N. and T.G.M. wrote the paper.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

### Appendix S1. Detailed description of methodology.

**Fig. S1.** The annual probability of a mass mortality event (>1% mortality of total population) under different proportions of habitat forest cover on the wintering grounds over time.

**Fig. S2.** Daily minimum temperature used to describe temperature patterns at the overwintering colonies in Mexico between December and March.

**Table S1.** Key to notation used in the spatial periodic matrix model for migratory monarch butterflies (*Danaus plexippus*) in eastern North America.

**Table S2.** Sample sizes of monarch butterflies captured in each region for assigning migratory connectivity (Flockhart *et al.* 2013).

**Table S3.** Monthly transition of monarch butterflies between destination region where they were captured and their region of origin based on stable isotopes.

**Table S4.** Survival of monarch butterflies during migration.

**Table S5.** Data used in the Geographic Information System used to calculate milkweed abundance in eastern North America.

**Table S6.** Land-cover classification.

**Table S7.** Milkweed density ( $\text{m}^2 \text{ha}^{-1}$ ) for different land-cover types in eastern North America.

**Table S8.** Results of models used to explain road and right-of-way widths in eastern North America.

**Table S9.** The width of roads in eastern North America.

**Table S10.** The width of road right-of-ways in eastern North America.

**Table S11.** Transition matrix of annual land-cover change based on data between 1982 and 2007.

**Table S12.** The proportion of total row crops grown as corn and soybean among the three breeding regions in eastern North America.

**Table S13.** Parameter estimates used in a logistic regression to predict changes in the adoption rates of genetically modified corn and soybean crops over time.

**Table S14.** Monthly weather in monarch butterfly overwintering colonies in Mexico.

**Table S15.** Future monthly mean minimum temperatures in monarch butterfly overwintering colonies in Mexico.

**Table S16.** Annual rates of winter habitat degradation between 1971 and 2012 in Oyamel fir-pine forest ecosystems, Mexico.





Docket No. APHIS-2013-0042

Regulatory Analysis and Development, PPD, APHIS  
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11 March 2014

**Comments to USDA APHIS on Dow AgroSciences LLC; Draft Environmental Impact Statement for Determination of Nonregulated Status of Herbicide Resistant Corn and Soybeans**

**Center for Food Safety, Science Comments II**

By: Martha L. Crouch, Ph.D., Science Consultant for CFS

These comments submitted by Center for Food Safety are one of two sets of science comments from our organization. Legal comments are also being submitted. The references cited have been uploaded as supporting materials. The filenames for these documents match the citations in the text, and are all incorporated as (e.g. Benbrook 2012). Full citations are included at the end.

These comments supplement and incorporate by reference our earlier two rounds of comments on the draft Environmental Assessments for event DAS-68416-4 (Enlist soybeans) and event DAS-40278-9 (Enlist corn), as well as all the references submitted previously. The previous CFS science comments on the draft EAs have been resubmitted as Appendix B. The previous CFS science comments will be cited in this text as “CFS Science Soy”, “CFS Science Corn I”, and “CFS Science Corn II”.

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## **Overview**

In its analyses of its proposed Plant Protection Act approval decision, APHIS fails to adequately consider, among other things, the effects on non-target organisms of approving Enlist corn and soybeans. Non-target organisms affected include, but are not limited to, plants growing within and near Enlist crop fields, wildlife such as migratory birds and butterflies, threatened and endangered species, and species beneficial to agriculture, such as pollinators, mycorrhizal fungi, nitrogen fixing bacteria, and predators of plant pests. APHIS does not consider impacts to these organisms from applications and off-target movement of herbicides used as part of the Enlist system. Nor does it consider the differences in potentially toxic herbicide metabolites between Enlist corn and soybeans and unmodified recipient organisms that may harm non-target organisms that consume or come in contact with Enlist corn and soybean plant parts.

## **APHIS excludes important analyses of herbicide impacts to non-target organisms based on invalid assumptions**

In its assessments, APHIS excludes impacts of applications and off-target herbicide movement, and impacts of herbicide metabolites, saying that only EPA has authority to regulate pesticides, and that EPA's regulation will mitigate any adverse impacts to health and environment. For example, APHIS defers to EPA's regulations to mitigate harm from herbicide use on Enlist corn and soybeans:

EPA is conducting an independent assessment of direct and indirect effects associated with the use of 2,4-D on DAS-40278-9 corn, DAS-68416-4 soybean, or DAS-44406-6 soybean concurrent with the development of this EIS. These effects are outside the scope of this EIS. APHIS decisions for the petitions for these new GE varieties will be made independent of the results of the EPA assessment. One assumption of the APHIS analysis is that EPA will establish label restrictions that will ensure the safety standards for human health and the environment associated with the use of Enlist Duo™ on these three varieties will be met. Therefore, APHIS' analysis in this section focuses on cumulative impacts associated with these varieties including the development of HR weeds due to herbicide application and changes in management practices resulting from their use. (EIS at 118, emphasis added)

APHIS explicitly states this and other assumptions when justifying its relegation of herbicide considerations to cumulative impacts of EPA's expected registration of Enlist Duo herbicide combined with approval of Enlist corn and soybeans (EIS at 118 - 119). Other assumptions by APHIS relevant to non-target organisms are:

APHIS assumes that herbicide applications will conform to the EPA-registered uses for corn and soybean that are summarized in Appendix 7 and 8. ...

APHIS assumes that drift from 2,4-D and other pesticide applications will be mitigated to an acceptable level by the registration requirements established by EPA. (EIS at 119, underline added)

APHIS does not provide evidence in support of these assumptions, however. In fact, there is

evidence to the contrary, as discussed by CFS in previous comments (CFS Science Soy at 43 – 45, 76 - 78 ), and below. APHIS’s reliance on these assumptions is thus contrary to sound science, and renders its conclusions arbitrary, since the agency refused to analyze important impacts of its proposed action.

**EPA’s label restrictions have not ensured safety standards for the environment from use of herbicides on previously approved resistant crops**

There is good evidence that EPA’s label restrictions have not “....ensure[d] the safety standards for human health and the environment associated with the use of...” herbicides with previously approved herbicide resistant crops. For example, glyphosate applications on glyphosate-resistant corn and soybeans, presumably used according to label instructions, has essentially eradicated common milkweed from fields in the Midwest (CFS Science Soy at 79 - 80). Common milkweed in corn and soybean fields is the most important food plant for monarch butterfly larvae in North America, producing almost 80% of the butterflies that overwinter in Mexico (Pleasants and Oberhauser 2012). Monarch populations have plummeted in recent years, with the lowest overwintering population ever recorded this year (Rendón-Salinas & Tavera-Alonso 2014), continuing an alarming 20-year decline of more than 90% (Brower et al. 2011, 2012), and raising concern that the entire migration is in jeopardy. Scientists have linked this dramatic decline in monarchs in large part to loss of breeding habitat from milkweed eradication by glyphosate use on glyphosate-resistant crops (Pleasants and Oberhauser 2012).

EPA’s label regulations failed to prevent this important harm to the environment, even though monarch biologists predicted the result soon after glyphosate-resistant crops were approved (e.g., Simpson 1999, Hartzler and Buhler 2000, Brower 2001). Now, in the EIS, APHIS has failed to assess impacts of approving Enlist corn and soybeans on monarchs, even after learning of harm from previous herbicide-resistant corn and soybean approval decisions, and seeing the evidence that EPA’s label restrictions were not protective. APHIS must consider how approval of Enlist corn and soybeans will impact milkweeds and monarchs, including associated use of herbicides, rather than improperly deferring responsibility for assessment to EPA (discussed in more detail below).

**EPA’s registration has not mitigated drift from herbicides to acceptable levels**

Also, in spite of EPA’s regulation, off-target herbicide movement, including drift of glyphosate applied on glyphosate-resistant crops, has resulted in many incidents where non-target organisms were harmed (US-EPA 2009). Glyphosate use has increased dramatically in concert with widespread adoption of glyphosate resistant crops (CFS Science Soy at 21 - 26). Even though glyphosate is not volatile, it nevertheless has become one of the most common herbicides detected in air and rain samples as fine droplets become airborne (Chang et al. 2011, Majewski et al. 2014). Glyphosate and its metabolites are also frequently measured in runoff and surface water (Battaglin et al. 2009, Coupe et al. 2012), glyphosate-resistant soybean samples (Bøhn et al. 2013), and in urine from both rural and urban people (Curwin et al. 2007a, 2007b). In other words, glyphosate is now practically ubiquitous in the environment. In some cases, glyphosate is measured at levels that can harm non-target organisms, such as amphibians (Relyea 2011) and plants (US-EPA 2009). Much of this glyphosate is likely to have originated in labeled applications to glyphosate-resistant crops (Coupe et al. 2012, Majewski et al. 2014). Many



people find this level of off-target movement, including drift, to be unacceptable (for example, growers whose crops have been injured: CFS Science Soy at 43 – 44). APHIS does not provide evidence that off-site movement of 2,4-D used with Enlist corn and soybeans will be mitigated by EPA's regulations any more effectively, and its assumption to the contrary is belied by past crop experiences and sound science. In fact, 2,4-D's volatility makes off-site movement even more prevalent and APHIS's reliance on EPA further misplaced.

**Not all herbicide applications to HR crops in the past have conformed to EPA's requirements**

APHIS' assumption that herbicide use on Enlist corn and soybeans will always conform to EPA-registered uses as described in Appendix 7 and 8, where APHIS describes what label it assumes EPA will require, is also unfounded, because it is contrary to experience with previously approved herbicide-resistant crops. There are well known examples of off-label applications of herbicides to resistant crops in certain circumstances where growers find benefits (CFS Science Corn II at 36: use of glufosinate on WideStrike cotton), and APHIS has not analyzed the conditions under which off-label use is likely to occur with Enlist corn and soybeans in order to assess risks. Also, herbicides are sometimes applied when environmental conditions are not as required on the label (AAPCO 2002).

**APHIS arbitrarily considers herbicide impacts from 2,4-D use on Enlist corn and soybeans, but refuses to consider these impacts in other contexts**

Although it claims that direct and indirect effects of 2,4-D use on Enlist corn and soybeans are outside of the scope of the EIS, APHIS nevertheless considers impacts of herbicide use with Enlist corn and soybeans when assessing socioeconomic impacts of increased weed resistance to those herbicides – a harm that only will occur if the herbicides are registered and used on Enlist crops:

Because of the likely adverse socioeconomic impacts that would result in the event that 2,4-D resistant weeds would be selected from the expected increased 2,4-D use on Enlist™ crops, APHIS believed these impacts may be significant. Therefore APHIS concluded that, for the three Enlist™ varieties that are the subject of this EIS, it will, at its discretion, prepare an EIS to further analyze the potential for selection of 2,4-D-resistant weeds and other potential impacts that may occur from making determinations of nonregulated status for these varieties. This EIS limits its analysis of herbicide use to the cumulative impacts that occur from the selection of herbicide resistant weeds and the changes in management practices that result. (EIS, at xi)

Limiting the scope of its EIS to cumulative impacts from 2,4-D selection of resistant weeds and resulting agricultural practices when there are many other direct, indirect and cumulative impacts of herbicide use with Enlist corn and soybeans, including to non-target organisms, is arbitrary and contrary to sound science.

Impacts of the APHIS approval of Enlist corn and soybeans must be assessed by APHIS under realistic scenarios, considering all reasonably foreseeable factors. Neither APHIS nor Dow provides any reason that a farmer would buy and plant Enlist crops unless he or she planned to use 2,4-D and glyphosate on those fields, since the engineered traits confer no advantage in

environments where the herbicides are absent.

In fact, APHIS fully expects Enlist Duo pre-mixed herbicide formulation containing 2,4-D and glyphosate to be registered by EPA for use on the corn and soybeans that are being considered in this EIS (see, for example, EIS at ix: “If APHIS approves the three petitions for nonregulated status for Enlist™ corn and soybean, it is reasonably foreseeable that EPA will independently approve registration of Enlist Duo™ herbicide for use on these GE plant varieties.”). APHIS has stated this expectation in the EIS (at ix, and elsewhere), and has been working closely with EPA to coordinate actions related to its approval. Therefore, analyses of its approval action and considering “alternatives” that do not take into account the use of 2,4-D and glyphosate are inappropriately based on an obviously unrealistic scenario where no 2,4-D is applied (see, for example, EIS at 108, where APHIS states there will be no direct or indirect effects of approval on agronomic practices and costs of production, because “...currently growers cannot use any herbicides differently on Enlist™ corn or soybean than are available to them for other corn and soybean varieties. That is because Enlist Duo™ is not registered for use on these corn or soybean events until EPA approves the label.”).

**Impacts to non-target organisms of applications of herbicides on Enlist corn and soybeans must be considered**

Herbicide use on Enlist corn and soybeans may harm non-target species within and around those fields (CFS Science Soy at 76 – 93, CFS Science Corn II at 12 - 25), and must be considered by APHIS in its assessments. APHIS does admit that herbicide use in agriculture impacts biodiversity (EIS at 143), as part of its cursory look at cumulative impacts. However, APHIS relies on a few industry-associated reviews instead of the large body of independent, peer-reviewed primary studies and reviews that are available on impacts of agricultural practices on biodiversity, so does not base its assessment on sound science. For example, there are many recent reviews and studies of impacts to biodiversity of organic agriculture compared with other agricultural regimes (e.g., Andersson et al. 2012, Blaauw and Isaacs 2012, Gaba et al. 2013, Gabriel and Tschamntke 2007, Hyvonen and Huusela-Veistola 2008, Kennedy et al. 2013, Kremen and Miles 2012, Lynch 2012, Morandin and Winston 2005, Nicholls and Altieri 2012, Power et al. 2012, de Snoo et al. 2013, Tuck et al. 2014).

In addition, APHIS skirts the impacts of the specific herbicides that will be used on Enlist crops, saying that herbicide use cannot be predicted:

Herbicide use in agricultural fields can impact biodiversity by decreasing weed quantities or causing a shift in weed species. This can affect insects, birds, and mammals that use these weeds. The quantity and type of herbicide use associated with conventional and GE crops depends on many variables, including cropping systems, type and abundance of weeds, production practices, and individual grower decisions. (EIS at 143)

Elsewhere, APHIS does predict that 2,4-D use will increase dramatically with adoption of Enlist corn and soybeans. Impacts of this APHIS approval-associated increase in the specific herbicide 2,4-D, and the other herbicides Enlist corn and soybeans were engineered to withstand, must be assessed, rather than waved away by claims that quantity and type of herbicides used are too variable to predict.

For example, APHIS does not assess impacts of increased use of 2,4-D combined with glyphosate on monarch butterflies, even though this important non-target species is already impacted by herbicide use with herbicide-resistant corn and soybeans (discussed below).

Impacts of glufosinate use on Enlist soybeans must also be analyzed by APHIS. Glufosinate is a potent broad-spectrum herbicide, toxic to non-target crops and wild plants at low levels via drift and runoff of water and soil (Carpenter and Boutin 2010, EPA EFED Glufosinate 2013). Therefore glufosinate use on Enlist soybeans will impact non-target crops and wild plants, including threatened and endangered plants, with consequences for biodiversity. In addition, glufosinate is directly toxic to some animals at environmentally relevant concentrations. Beneficial insects may be particularly at risk from glufosinate use on Enlist soybeans, including predatory mites and spiders, and lepidopteran pollinators (discussed below). Mammals present in the agroecosystem may experience chronic toxicity. Pest and pathogen levels may be altered. Also, threatened and endangered animals may be put at greater risk by glufosinate use on Enlist soybeans. These are significant adverse impacts that APHIS must assess and meaningfully consider in its assessments.

In addition, APHIS fails to analyze impacts of quizalofop use on Enlist corn, even though Enlist corn is engineered to resist this herbicide via the same enzyme that confers resistance to 2,4-D.

#### **APHIS does not consider pests and pathogen impacts of herbicide use with Enlist corn and soybeans**

CFS commented on potential pest and pathogen impacts of herbicides used with Enlist soybeans to crops and non-target organisms (CFS Science Soy at 44), concluding that drift of 2,4-D can cause symptoms similar to injury from pests and pathogens, and herbicides can suppress or stimulate pests and pathogens, as well.

In addition, glufosinate has been shown to affect various plant pathogens, both after applications to resistant crops, and in culture (reviewed in Sanyal and Shrestha 2008). Some effects of glufosinate on pathogens may be beneficial for agriculture, and some may be harmful. In glufosinate-resistant rice, glufosinate has been shown to trigger transcription of pathogenesis-related genes and other defense systems that act in concert with direct suppression to protect the GE rice from blast and brown leaf spot diseases (Ahn 2008). In contrast, glufosinate may be harmful to agriculture by suppression of pathogens of weeds and pests, allowing those weeds and pests to cause more damage.

Therefore, APHIS must consider the changes in pests and pathogens of non-target plants as a result of increased herbicide use and different patterns of herbicide use resulting from approval of Enlist corn and soybeans, and it does not do so in the EIS.

#### **APHIS does not adequately consider risks to species beneficial to agriculture**

##### ***Beneficial microorganisms***

Beneficial microorganisms include species in the rhizosphere of corn and soybeans, and on leaf and stem surfaces that mediate nutrient relationships, diseases, and environmental stresses. Also,

soil microbes are involved with decomposition, nutrient cycling, and other functions (Cheeke et al. 2013).

APHIS describes in general terms the importance of microbes in agricultural soils. Herbicide use is one factor APHIS identifies as influencing microbial populations:

The main factors affecting microbial population size and diversity include: (1) the plant species, cultivars, and developmental stages present, which provide specific carbon and energy inputs into the soil; (2) soil type (determined by texture, structure, organic matter, aggregate stability, pH, and nutrient content); (3) geographic location; (4) season; (5) weather; (6) agricultural management practices (crop rotation, tillage, herbicide and fertilizer application, and irrigation) (Young and Ritz, 2000; Kowalchuk *et al.*, 2003; Garbeva *et al.*, 2004). (EIS at 37)

Even though APHIS acknowledges that herbicides can affect microbes, the impacts on soil microbes of the specific changes in herbicide use as a result of its proposed approval of Enlist corn and soybeans are not considered by APHIS.

Two classes of microorganisms that are particularly beneficial to soybean production are nitrogen-fixing bacteria and mycorrhizal fungi, as APHIS acknowledges:

An important group of soil microorganisms associated with legumes, including soybean, are the mutualists. These include mycorrhizal fungi, nitrogen-fixing bacteria, and some free-living microbes that have co-evolved with plants that supply nutrients to and obtain food from their plant hosts (USDA-NRCS, 2004). Legumes have developed symbiotic relationships with specific nitrogen-fixing bacteria in the family *Rhizobiaceae* that induce the formation of root nodules where bacteria may carry out the reduction of atmospheric nitrogen into ammonia (NH<sub>3</sub>) (NRC, 2004). *Bradyrhizobium japonicum* is the rhizobium bacteria specifically associated with soybeans (Franzen, 1999). (EIS at 99)

In fact, most soybean growers do not apply nitrogen fertilizers (EIS at 79), since usually all nitrogen needed for plant growth is obtained from the association of soybeans with symbiotic rhizobia and nitrogen already available in the soil (Ruark 2009).

Enlist soybeans are the first broadleaved plant that will be sprayed directly with 2,4-D, and also the only genetically engineered crop that harbors symbiotic nitrogen-fixing bacteria. Therefore, it is crucial that APHIS analyzes and assesses risks to rhizobium and the nitrogen fixation process in Enlist soybeans under realistic field conditions that include herbicides that Enlist soybeans have been engineered to withstand. APHIS does not analyze or assess impacts of 2,4-D as used on Enlist soybeans in any specific way, nor does Dow provide any specific data or observations on nitrogen fixation in Enlist soybeans with or without associated 2,4-D use.

Enlist soybeans are also glufosinate resistant. Some studies have shown negative effects of glufosinate on beneficial microbes. Pampulha et al. (2007) treated soil in laboratory microcosms with the glufosinate formulation “Liberty” at different concentrations and durations, and then determined the types, numbers and functional activity of culturable microorganisms – bacteria,



fungi, and actinomycetes; cellulolytic fungi, nitrite oxidizing bacteria, and dehydrogenase activity. They found a complex pattern of changes in number and activity of microbes. However, the most dramatic change in response to glufosinate was a large decrease in dehydrogenase activity over time, which they say is a good indicator of general microbial activity. They conclude that glufosinate use “may have injurious effects on soil microorganisms and their activities.”

APHIS does make a general statement that “[s]everal reviews of the investigations into the impact of GE plants on microbial soil communities found that most of the studies examining distinctive microbial traits concluded that there was either minor or no detectable non-target effects...” (EIS, at 99). In fact, glyphosate use on glyphosate-resistant soybeans has been shown to impair nitrogen-fixing bacteria in some circumstances (Zablotwicz and Reddy 2007, Kremer and Means 2009, Zobiole et al. 2010, Bohm et al. 2009). And, more importantly, none of these reviews include studies of use of 2,4-D on GE, resistant soybeans, or use of 2,4-D on any GE crop.

If approval of Enlist soybeans does lead to a reduction in nitrogen fixation in soybeans, then soybean growers may need to add more nitrogen fertilizer to their fields, with increased socioeconomic costs and environmental impacts. Impacts on nitrogen fixation need to be ascertained before concluding, as APHIS does, that agronomic inputs will not be changed by a deregulation decision (EIS, p. 121).

### ***Beneficial fungi***

Impacts of the approval of Enlist corn and soybean interactions with beneficial fungi also are not specifically considered by APHIS. Both corn and soybeans benefit from being infected by mycorrhizal fungi that live in their roots. These fungi facilitate movement of nutrients from the soil, protect against pathogens, and moderate effects of drought (Harrier and Watson 2003, Cheeke et al. 2013: Chapter 7). A wide range of agronomic practices influences the numbers and kinds of mycorrhizal fungi. Studies have even shown that corn varieties genetically engineered with insect-resistant Bt traits inhibit mycorrhizae in certain conditions (Cheeke et al. 2013: Chapter 8), possibly due to changes in root exudates. APHIS must assess impacts of its proposed approval of Enlist corn and soybeans on mycorrhizal fungi under realistic field conditions covering a range of stresses that these fungi are known to ameliorate, and that include applications of the herbicides Enlist soybeans have been engineered to withstand.

### ***Predators of crop pests***

Predators of crop pests may be harmed by use of herbicides on Enlist corn and soybeans, and this was not analyzed by APHIS in the EIS. For example, glufosinate is toxic via a metabolic pathway found in animals and microorganisms, as well as plants, and some animals are injured or killed by herbicidal doses (EPA EFED Glufosinate 2013). Arachnids such as mites and spiders are particularly sensitive to glufosinate.

Although some mite species are serious agricultural pests of many crops, including corn, the use of pesticides for their control is not generally an effective strategy. Pesticides fail because many pest mites have developed resistance; while predatory mites, spiders and other insects that are important for keeping pest mite populations low are susceptible. Therefore, Integrated Pest

Management systems are recommended, where healthy predator populations are encouraged (Peairs 2010).

Glufosinate can harm predatory mites. Experiments on the direct toxicity of various pesticides to a predator mite found in Virginia vineyards showed glufosinate to be particularly toxic, causing 100% mortality within a day (Metzger and Pfeiffer 2002). Although the dose used was greater than that for resistant corn, lower doses were not tested.

Further experiments on glufosinate and beneficial arthropods were carried out in conjunction with a risk assessment by the European Food Safety Authority (EFSA 2005), and included glufosinate applications as used on corn:

The European Food Safety Authority (EFSA 2005) evaluated a series of extended laboratory and semi-field studies on beneficial insects including the parasitoid wasp (*Aphidius rhopalosiphi*), predatory mite (*Typhlodromus pyri*), wolf spider (*Pardosa* ssp.), green lacewing (*Chrysoperla carnea*), ground beetle (*Poecilus cupreus*), and rove beetle (*Aleochara bilineata*). “Severe” effects were observed with a potential for population recovery in one season when glufosinate was applied at rates consistent with use on glufosinate-resistant corn (two application at 0.8 kgai/ha) (EPA EFED Glufosinate 2013 at 95)

Although there was “potential for population recovery in one season”, the risks to beneficial insects were considered to be high enough to warrant mitigation:

As described in the EFSA (2005) report, the EFSA Peer Review Coordination (EPCO) expert meeting (April 2004, ecotoxicology) recommended mitigation measures for risk to nontarget arthropods, such as a 5-m buffer zone when glufosinate is applied to corn or potatoes. (EPA EFED Glufosinate 2013 at 95).

Data from EPA also indicates that large buffers may be required to protect non-target terrestrial plants from injury (EPA EFED Glufosinate 2013 at 98), and thus reduce harm to non-target predatory mites and spiders, and other beneficial arthropods.

### **Beneficial mammals**

Some mammals are considered beneficial to agriculture, including corn and soybeans. For example, some rodents eat weed seeds, reducing the weed seed bank (EFSA 2005), or become food for predators that control pest species. Other mammals are predators of corn and soybean pests.

APHIS does not analyze risks to beneficial mammals from the use of 2,4-D with Enlist corn and soybeans, even though APHIS includes information from EPA in Appendix 8. Both acute and chronic risks to mammals have been identified by EPA in screening level risk assessments for the 2,4-D use patterns being planned for Enlist corn and soybeans (EIS at 8-10 appendix). EPA also identified indirect risks to mammals from modification of their habitat by 2,4-D use with Enlist crops (EIS at 8-10). CFS has commented on risks from 2,4-D use to mammals and other animals, as well (CFS Science Soy at 83).

Glufosinate use on Enlist corn and soybeans is likely to exceed levels of concern for chronic risk to mammals that eat insects, and plant parts other than strictly fruits, seeds and grains (EPA EFED Glufosinate 2013 at 70), as summarized:

The screening level assessment with preliminary refinements concludes that the use of glufosinate in accordance with registered labels results in chronic risk to mammals that exceeds the Agency's chronic risk Level of Concern (LOC). Adverse effects in mammals following chronic exposure to glufosinate in laboratory studies include reductions in growth and in offspring fitness and viability; these effects are seen across generations and in multiple species (EPA EFED Glufosinate 2013 at 5).

Chronic effects of glufosinate at the expected exposure levels in laboratory studies “include reductions in parental and offspring growth and offspring viability. These effects have been observed in multiple studies and have been shown to extend to the second generation (no subsequent generations were tested).” (EPA EFED Glufosinate 2013 at 92)

Formulated products are more acutely toxic to mammals than the active ingredient alone by an order of magnitude (EPA EFED Glufosinate 2013 at 91), and formulations may also cause chronic toxicity at lower levels.

EFSA identified a high risk to mammals from glufosinate use in glufosinate-resistant corn based on chronic toxicity, and considered it to be “critical area of concern” (EFSA 2005).

### **Pollinators**

Pollinators are beneficial to agriculture. Even though corn is wind-pollinated, and soybeans are mainly self-pollinating, pollinators necessary for other crops and wild plants are known to collect pollen from corn and nectar from soybeans (Krupke et al. 2012), and pollinators use the other plant species found within and around corn and soybean for food and other habitat requirements. Thus APHIS must assess the impacts on pollinators of herbicide use with Enlist corn and soybeans, but they did not do so in the EIS.

CFS discussed impacts on pollinators of 2,4-D use with Enlist corn and soybeans are at length (CFS Science Corn II at 35 – 41, and below in relation to nectar plants used by monarchs.

Glufosinate use with Enlist soybeans may have direct effects on lepidopteran (butterfly and moth) pollinators when larvae eat glufosinate-containing pollen, nectar or leaves, either after direct over-spray or from drift. Laboratory experiments with the skipper butterfly *Calpodesthlias* showed that larvae fed glufosinate-coated leaves were injured or killed by inhibition of glutamine synthase, at doses “comparable to the amount that might realistically be acquired by feeding on GLA [glufosinate]-treated crops.” These studies were done with the active ingredient, not a full formulation, and so may have underestimated field toxicity (Kutlesa and Caveney 2001).

Nectar of glufosinate-treated Enlist soybeans may accumulate significant levels of glufosinate. Although primarily a contact herbicide, glufosinate does translocate via phloem to a limited

degree, depending on the plant species (Carpenter and Boutin 2010). In experiments comparing glufosinate translocation in GE resistant canola versus a susceptible variety (Beriault et al. 1999), glufosinate translocated more readily in resistant plants. However, in both resistant and susceptible canola, glufosinate moved in the phloem to developing anthers without causing injury to tissues along the way. If glufosinate is retained in leaves of resistant soybeans, it may translocate to nectar later, even if the applications occur well before flower formation.

APHIS should examine data on glufosinate levels in flowers of Enlist soybeans after labeled applications to assess risks to beneficial pollinators.

Pollinators may also be affected by changes in habitat from glufosinate toxicity to plants. Numbers and kinds of plants can change dramatically in response to herbicide applications, with impacts that ripple through ecosystems (as discussed in previous CFS comments, and in relation to monarchs, below). In addition, pollinators that depend on specific host plants may be affected if those plants are more sensitive to glufosinate (Pleasants and Oberhauser 2012).

Large buffers may be required to protect non-target terrestrial plants from injury (EPA EFED Glufosinate 2013 at 98), and thus reduce harm to pollinators.

APHIS also does not consider impacts of quizalofop use on corn to pollinators in the EIS.

**Risks to monarch butterflies from herbicide use associated with approval of Enlist corn and soybeans are not assessed by APHIS**

The recent decline of monarchs (*Danaus plexippus*) is a clear example of harm to a non-target organism from past APHIS approval of herbicide-resistant corn and soybeans, as CFS commented (CFS Science Soy at 79 -80), yet APHIS does not analyze impacts to monarchs of approving Enlist corn and soybeans in the EIS.

Monarch numbers in North America are at their lowest since records have been kept, and biologists are concerned that the monarch migration is in jeopardy (Brower et al. 2011, 2012). At their most recent peak in 1997, there were almost a billion monarch butterflies overwintering in oyamel fir trees in the central mountains of Mexico (Slayback et al. 2007). This year, counts indicate an overwintering monarch population of fewer about 33 million, by far the lowest ever measured (WWF-Mexico 2014), continuing an alarming 20-year decline of more than 90% (Brower et al. 2011, 2012).

Although there are many factors at play, scientists have shown that a critical driver of the recent steep decline in monarch butterfly numbers is loss of larval host plants in their main breeding habitat, the Midwest corn belt of the US, as CFS commented previously (CFS Science Soy at 79-80, Pleasants and Oberhauser 2012). Monarchs lay eggs exclusively on plants in the milkweed family, and the larvae that hatch from these eggs must consume milkweed leaves to complete the butterfly's lifecycle (Malcolm et al. 1993). Common milkweed has been largely eradicated from corn and soybean fields where it used to be common (Hartzler 2010, Pleasants and Oberhauser 2012), depriving monarchs of the plant they require for reproduction.



**Glyphosate used with glyphosate-resistant corn and soybeans has removed common milkweed from corn and soybean fields, decimating the monarch population**

Common milkweed (*Asclepias syriaca*) is a perennial plant with shoots that die back in the winter, but re-sprout from buds on spreading roots in the spring to form expanding colonies (Bhowmik 1994). Common milkweed also regrows when the plants are mowed, chopped by tillers, or treated with many kinds of herbicides that only kill aboveground plant parts, or are applied before milkweed shoots emerge in late spring (Bhowmik 1994). Thus, until recently, common milkweed has been found within and around corn and soybean fields in sufficient numbers to support a large population of monarch butterflies. In fact, in the late 1990s when monarch numbers were still high, almost half of the monarchs in Mexican winter roosts had developed on common milkweed plants in the Midwest corn belt, making this the most important habitat for maintaining the monarch population as a whole (Wassenaar and Hobson 1998).

Recently, though, the widespread adoption of genetically engineered, glyphosate-resistant corn and soybeans has triggered a precipitous decline of common milkweed, and thus of monarchs (Pleasants and Oberhauser 2012). Glyphosate, is one of the extremely few herbicides that efficiently kills milkweed (Waldecker and Wise 1985, Bhowmik 1994). Glyphosate moves throughout the plant – from sprayed leaves into roots, developing shoots and flowers – where it thwarts milkweed’s reproductive strategies.

Glyphosate is particularly lethal to milkweed when used in conjunction with glyphosate-resistant corn and soybeans (patterns of glyphosate use on resistant crops are described in detail in CFS Science Soy at 6, 14 – 15, 21- 24). It is applied more frequently, at higher rates, and later in the season (during milkweed’s most vulnerable flowering stage of growth) than when used with traditional crops. The increasingly common practice of growing glyphosate-resistant corn and soybeans every year means that milkweed is exposed to glyphosate every year without respite, and has no opportunity to recover. In fact, in the 15 years since glyphosate-resistant soybeans, and then corn, were approved by APHIS, common milkweed has been essentially eliminated from corn and soybean fields in the major breeding area for monarch butterflies (Hartzler 2010).

This loss of habitat for monarch butterflies, because of eradication of the only host plant that grows within corn and soybean fields in the Midwest, has been devastating. Fewer corn and soybean fields have milkweed plants, and where they do occur, the plants are more sparsely distributed. In a 1999 survey of Iowa, common milkweed was found in half of corn and soybean fields, and this milkweed occupied an aggregate area of almost 27,000 acres (Hartzler and Buhler 2000). A decade later in 2009, a second survey found that only 8% of corn and soybean fields had any milkweed plants at all, with an aggregate area of just 945 acres – a 96.5% decline (Hartzler 2010). By 2012, it is estimated that just over 1% of common milkweed remained in corn and soybean fields in Iowa compared to 1999, just a few hundred combined acres (extrapolated from Pleasants and Oberhauser 2012). It is clear that other Midwestern states have experienced similarly devastating milkweed losses, based on comparable land-use patterns and other evidence.

Rapid, large-scale changes in glyphosate use (e.g. Benbrook 2009, as cited in CFS Science Soy) are responsible for milkweed loss. Common milkweed in corn and soybean fields has been unable to survive the change in glyphosate use that accompanied approval of glyphosate-resistant

corn and soybeans (Pleasants and Oberhauser 2012).

Milkweeds do still remain outside of agricultural fields in the Midwest, but there aren't enough of them to support a viable monarch population. The combined area of roadsides, Conservation Reserve Program (CRP) land, and pastures is only about 25% of corn and soybean acreage in Iowa, which is representative of the Corn Belt as a whole (Pleasants and Oberhauser 2012). In addition, monarchs produce almost four times more progeny per milkweed plant in corn and soybean fields than in non-agricultural areas (Monarch Larval Monitoring Project, as described in Pleasants and Oberhauser 2012), so agricultural milkweed is more valuable as habitat. Thus, even if non-crop lands have a higher density of milkweeds, they cannot begin to compensate for agricultural habitat lost to glyphosate use on glyphosate-resistant corn and soybeans.

**Impact of APHIS approval of Enlist corn and soybeans on common milkweed will continue glyphosate harms in addition to new harms from Enlist-associated herbicide use**

As confirmed by APHIS in the EIS, Enlist corn and soybeans will be sprayed post-emergence with a pre-mix formulation of glyphosate and 2,4-D. In addition, they may be sprayed glufosinate or quizalafop. Farmers may also apply the individual herbicides sequentially.

Enlist corn and soybeans will therefore not only continue to be sprayed post-emergence with glyphosate, but also with other herbicides, when common milkweed is in its most vulnerable reproductive stages (Bhowmik 1994). Even those herbicides that are weaker on perennial weeds such as milkweed (e.g. glufosinate) can be expected to cause considerable damage to aboveground plant parts. In addition, Enlist corn and soybeans are engineered to be extremely resistant to the herbicides in question, enabling application of rates higher than have ever been used before without injuring the crop. Herbicides that cause limited damage to weeds when applied at lower rates are often much more damaging at higher rates. The combination of additional active ingredients applied post-emergence, and use of higher rates, can only accelerate the demise of common milkweed in corn and soybean fields while preventing its reestablishment, especially in view of the fact that glyphosate will continue to be used at rates similar to those used at present on crops resistant to glyphosate alone.

***Efficacy of 2,4-D at killing common milkweed***

2,4-D is in the synthetic auxin class of herbicides. Synthetic auxins are generally effective on perennial broadleaf weeds because they, like glyphosate, are translocated to the root. 2,4-D and dicamba are the auxin herbicides most frequently recommended for control of common milkweed, though neither is as consistently effective as glyphosate.

The Ohio State University extension service recommends a high rate of glyphosate (2.25 lbs. a.e./acre) as the first option for control of common milkweed in non-crop or fallow field situations, but also notes that a lower rate of glyphosate (1.5 lbs ae/acre) combined with 2,4-D “can provide good control as well.” Likewise for corn, a post-emergence application of glyphosate is recommended if the corn is Roundup Ready. For non-Roundup Ready corn, dicamba is the top choice – alone or combined with one of several other herbicides (Ohio State Extension, as cited in Isleib 2012).

North Dakota State University has conducted tests evaluating the efficacy of various herbicides on common milkweed (Martin and Burnside 1984, Cramer and Burnside 1981). A high rate of glyphosate (3 lbs./acre) provided the best milkweed control when evaluated the following spring. Higher than normal rates of 2,4-D (2 lbs./acre) provided lesser but still considerable levels of control, reducing milkweed stands by roughly half.

Other studies on herbicidal control of common milkweed reveal quite variable results for 2,4-D (Cramer & Burnside 1981, Bhowmik 1982). In greenhouse experiments conducted by Cramer and Burnside (1981), 2,4-D provided modest suppression of common milkweed regrowth when evaluated five weeks after application, suppression almost equal to that of glyphosate (Cramer and Burnside, Table 1). Mixtures of glyphosate and 2,4-D were one of the most effective herbicide combinations (Table 1).

Field studies designed to assess the long-term efficacy of various herbicides on common milkweed generally show that 2,4-D did not provide much control in the year or two following a single application (Bhowmik 1982). However, these experiments generally involved low rates of 2,4-D and/or application in the fall when milkweed was past its reproductive phase (post-flowering), and so presumably less susceptible to herbicidal control.

Cramer and Burnside (1981) were unable to explain the variable efficacy exhibited by 2,4-D (or that of other herbicides) in the experiments they conducted, noting merely that herbicidal control of common milkweed “is variable ... and appears to be dependent on growth stage, growth rate, time of herbicide application, climatic variables, and other factors.”

#### ***Effects of Enlist Duo used with Enlist corn and soybeans on common milkweed***

The discussion above shows that 2,4-D suppresses common milkweed. Although not consistently as effective as glyphosate, particularly for longer-term control, its efficacy is regarded as sufficient to merit recommendations for its use on common milkweed by experienced agronomists at several universities.

Enlist corn and soybeans will greatly exacerbate the negative impacts of 2,4-D on common milkweed for several reasons: higher rates will be used; most applications will occur during milkweed’s most vulnerable reproductive phase; most applications will be in combination with glyphosate; much more cropland will be sprayed; and the frequency of use will increase both within season and over years (CFS Science Soy at 78).

Combined use of two herbicides known for their efficacy in killing milkweed can only hasten its eradication from crop fields and maintain its absence, with devastating consequences for monarch butterflies. APHIS does not consider these impacts of Enlist corn and soybean approval on monarchs in its EIS.

#### **Herbicide drift injury from Enlist corn and soybean fields to nectar plants**

Although monarch larvae are selective about food plants, only thriving on milkweeds, the adult butterflies derive nutrients from a wide variety of nectar-producing flowers (Tooker et al. 2002). They depend on flowers that are in bloom in their breeding habitat during the spring and

summer, and then along migration routes to winter roosts (Brower and Pyle 2004). Monarchs that are breeding during spring and summer use energy derived from nectar for flying, laying eggs, mating, and other activities. In addition, the generation that migrates in the fall converts nectar sugars into storage lipids to fuel their metabolism during winter, and perhaps also for northern migration the following spring (Brower et al. 2006).

Herbicides are toxic to plants, by definition, and their use in agricultural landscapes has resulted in changes in flowering plant populations within and around crop fields, with impacts felt throughout ecosystems. It has been shown that “[b]etween 5% (commonly) and 25% (occasionally) of the applied herbicide dose is expected to reach the vegetation in field margins and boundaries (e.g. hedgerows, woodlots, etc.) (Holterman et al., 1997; Weisser et al., 2002).” (Boutin et al. 2014).

There have been no surveys of wildflowers in agricultural landscapes before and after commercialization of previously approved herbicide-resistant crops, as important as such information is for assessing environmental impacts. However, glyphosate from use on herbicide resistant crops may have already reduced abundance and diversity of nectar plants in and around agricultural fields, from direct applications as well as spray drift (e.g. Gove et al. 2007, Blackburn and Boutin 2003). Approval of Enlist corn and soybeans that are associated with use of highly active, volatile 2,4-D with an even greater potential for causing drift injury, in addition to glyphosate, is likely to have severe impacts on nectar resources used by monarchs and other pollinators (Brower et al. 2006).

Hugely increased spray drift, volatilization and runoff from the much greater use of herbicides with Enlist corn and soybeans are likely to alter the very habitats important for biodiversity in agroecosystems, such as hedgerows, riparian areas, unmanaged field margins, and other areas where wild organisms live near fields (Freemark and Boutin 1995, Boutin and Jobin 1998, Olszyk et al. 2004). These areas harbor nectar plants for adult monarchs as well as milkweeds for larvae. Based on experiences with 2,4-D sensitive crops, for example, natural areas miles from agricultural applications of these herbicides will be at increased risk from the use of greater amounts on herbicide resistant crops, since these herbicides can volatilize under certain conditions (CFS Science Soy at and also come down in rain (Hill et al. 2002). Also, as CFS has commented, herbicides used on resistant crops are applied over a longer span of the growing season, and thus overlap a wider range of developmental stages of nearby plants, hitting them when they may be more sensitive to injury.

***Plants of different species and growth stages vary in sensitivity to herbicides, putting monarchs and other pollinators at risk***

Particular species of plants are more or less sensitive to specific herbicides (Olszyk et al. 2013, Boutin et al. 2004), and at different growth stages (Carpenter and Boutin 2010, Boutin et al. 2014), so that exposure can change plant population dynamics in affected areas. 2,4-D and other auxin-like herbicides such as dicamba are particularly potent poisons for many species of plants (Rasmussen 2001, US-EPA 2009), especially dicotyledons (broadleaf plants) that are sensitive to very low drift levels. Even monocots such as members of the grass and lily families can be killed by higher doses of 2,4-D or dicamba, and suffer sub-lethal injuries from drift levels at certain times in their life cycles (US-EPA 2009; Nice et al. 2004).



Plants – both crop and wild species – are often very sensitive to herbicide injury as flowers and pollen are forming (Olszyk et al. 2004). This has been clearly shown with dicamba and injury to tomato plants (Kruger *et al.* 2012) and soybeans (Griffin et al. 2013), and with glyphosate injury to rice flowers (Wagner 2011). Drift levels of dicamba have also been shown to affect asexual reproduction in potatoes (Olszyk et al. 2010), and seed production in peas (Olszyk et al. 2009), sometimes without accompanying vegetative injury. Glyphosate drift to potato plants has been responsible for causing potato shoots arising from seed potatoes in the next generation to grow abnormally or not at all (Worthington 1985), without always affecting the growth of the potato plants that were actually hit with the herbicide (Potato Council 2008). There are many other examples of differential sensitivity to particular herbicides (Boutin et al. 2014). Injury affecting flowers and vegetative propagules but not the rest of the plant can easily go undetected, nevertheless having a large impact on reproduction and thus subsequent generations.

Differential sensitivity to herbicides can lead to changes in species composition of plant communities. For example, as noted in CFS comments (CFS Science Soy at 81), 2,4-D movement away from crop fields in mid-spring may kill sensitive dicotyledonous wildflowers at seedling stages, cause male sterility in less sensitive grasses about to flower, and have little effect on younger grasses or still-dormant perennials (Olszyk et al. 2004). These impacts can cause long-term changes in the mix of plant species, favoring annual weeds and grasses over native plants and perennial forbs (broadleaved plants), for example (Boutin and Jobin 1998, Boutin et al. 2008). And if there are herbicide resistant plants in these habitats, they will of course be better able to withstand drift and may become more abundant (Watrud et al. 2011, CFS 2013a).

Pollinators are at particular risk from changes in plant populations and flowering behavior. Recently published comparisons of flowering plants in natural areas around fields that have been exposed to herbicides on a regular basis vs. near fields managed without herbicides show striking differences in abundance and kinds of plants in flower, and also in when these plants flower (Boutin et al. 2014). Hedgerows next to organic farms had more species, and many of them flowered earlier in the season and for a longer time span. These field observations confirmed greenhouse studies that showed significant delays in flowering of several species after exposure to herbicides (Boutin et al. 2014).

Such changes in which plants flower, and when, could affect monarchs as they breed and migrate, disrupting coordination between the butterflies and needed resources:

.... organic farming promoted not only plant diversity but also plant flowering capacity whereas conventional farming inhibited flower production of the fewer plants found in adjacent hedgerows and resulted in a shift in flowering. This in turn may cause disharmony with pollinator activities as pollinators can be very sensitive to flowering events (Santandreu and Lloret, 1999). Effects on timing of flowering can have consequences on pollinating insects as they may be less able to survive in non-crop habitats during periods when crop plants are unavailable for pollination (Carvalho et al., 2010). Alternatively, delays in flowering time may expose flowers to unfavourable weather conditions (e.g. frost or drought). Herbicide effects appear to constitute yet another stressor affecting plant – insect interactions, adding to other stressors including

land-use modifications at the landscape scale (Kremmen et al., 2007) that are increasingly impacting agro-ecosystems. (Boutin et al. 2014)

***Herbicides selective for broadleaved plants, such as 2,4-D, pose danger to nectar plants in particular***

Herbicides such as 2,4-D that selectively kill dicots may be particularly injurious to butterflies, often considered an indicator of ecosystem health. If these herbicides are applied frequently and over a broad area – as will happen with herbicide use on Enlist corn and soybeans– negative impacts on butterflies are likely to be increased. A study by Longley and Sotherton (1997) of pesticide effects on butterflies in agricultural areas of England makes this point:

The frequency and number of pesticide applications, the spatial scale of treatment and the degree of field boundary contamination during each spray occasion will determine the extent of damage to butterfly habitats and populations, and the rate at which populations will return to their original densities. (Longley and Sotherton 1997).

Researchers implemented experimental mitigation measures to determine whether changes in pesticide use would result in more butterflies in the landscape. One of these measure involved limiting the use of “persistent broadleaf herbicides” near field edges, and instead using herbicides that were more specifically targeted against grasses:

The outer section of a tractor-mounted spray boom (approximately 6 m) is switched off when spraying the outer edge of a crop, avoiding the use of certain chemicals (persistent broadleaf herbicides and all insecticides other than those used for controlling the spread of Barley Yellow Dwarf Virus). Whilst the rest of the field is sprayed with the usual compliment of pesticides, more selective chemicals (e.g. graminicides rather than broad-spectrum herbicides) are sprayed on the edges (Boatman and Sotherton, 1988). (Longley and Sotherton 1997, p. 8).

They found that there were indeed more butterflies after taking these measures, and also that there were more dicots, the main source of nectar, as well as more biodiversity in general:

In addition, as a result of selective herbicide use, Conservation Headlands are rich in broadleaved plants, thereby increasing the availability of nectar resources for butterfly species. (Longley and Sotherton 1997, p. 8)

The unsprayed headlands have also been shown to benefit the survival of rare weeds (Schumacher, 1987; Wilson, 1994), small mammals (Tew, 1988), beneficial invertebrates (Chiverton and Sotherton, 1991; Cowgill et al., 1993) and gamebird chicks (Rands, 1985; Rands, 1986). However, to be of long-term value for butterfly conservation, unsprayed headlands need to be maintained over consecutive years to allow the survival of those species which are univoltine and have poor powers of dispersal. (Longley and Sotherton 1997, p. 9)

In conclusion, these researchers emphasize the need for research on impacts of pesticide use over time:

In addition to short-term studies, covering single cropping seasons, information is also needed on the effects of different spray and cropping regimes over several seasons on butterfly communities in exposed areas. Only then will it be possible to make reliable predictions and recommendations for butterfly conservation on arable farmland. (Longley and Sotherton 1997, p. 12)

Implications of this butterfly study in England are clear for use of 2,4-D with Enlist corn and soybeans: 2,4-D is an herbicide that selectively kills broadleaved plants (dicots), the main nectar source for adult butterflies, even those species whose larvae feed on grasses. 2,4-D is also likely to be used more often during a season, more extensively in an area, and from year to year with Enlist corn and soybeans than it is currently used in agriculture. This is exactly the opposite use pattern than that recommended for mitigation of pesticide impacts on butterflies, that were also shown to be protective of biodiversity in general.

A new experimental study designed to test impacts of dicamba drift, an auxin-class herbicide and thus relevant to 2,4-D, on plant and arthropod communities in agricultural “edge” habitats highlights the importance of long-term studies of herbicide impacts over a range of environments (Egan et al. 2014). These researchers applied a range of doses of dicamba, meant to simulate different levels of drift, to field margins and to plots within old fields to determine whether plant and arthropod communities changed in response. In each habitat, they sprayed dicamba one time each year for two consecutive years, and performed plant censuses throughout the growing seasons, both before and after dicamba applications. In addition to monitoring the kinds and numbers of plants, number of flowers produced by each species was also recorded. For field margins, they also did a census of arthropods at different times during the growing season. Egan and colleagues found that low drift levels of dicamba did in fact affect plant and arthropod communities, but in complex ways, depending on plant successional status of the community to begin with, and environmental conditions such as water stress when herbicides were applied. However, impacts were seen at about 1% of the field application rate – a lower level than other studies have reported, and within the range expected to occur frequently from herbicide applications associated with herbicide-resistant crops. They advise:

In light of this variation across sites and environments, it is not possible to derive general predictions about how plants and arthropods will respond to non-target dicamba exposure. Further research is needed to better understand the species, communities, and habitat types that are most sensitive to dicamba drift and the environmental conditions during exposure that can moderate susceptibility. In the absence of predictive understanding, a precautionary emphasis on limiting non-target herbicide exposures is well-warranted. (Egan et al. 2014)

Similar cautions apply to 2,4-D use with Enlist corn and soybeans. By far the best way to limit herbicide exposure of important nectaring habitat for monarchs is to restrict post-emergence use of such herbicides.

#### **EPA regulations do not protect nectar plants from herbicide drift injury**

IEPA guidelines for protecting non-target plants from drift injury are based on toxicity tests that include too few species, tested at only a few points in their vegetative development, and

therefore underestimate the range of sensitivities in communities of wild species throughout their lifecycles (Pfleege et al. 2012, White and Boutin 2007, Olszyk et al. 2013, Boutin et al. 2014). These deficiencies in assessment of herbicide impacts will put the monarch's nectaring habitat at further risk should Enlist corn and soybeans be approved by APHIS.

**Monarchs may also be harmed by direct exposure to herbicides used with Enlist corn and soybeans**

Herbicides may directly harm exposed insects, such as monarchs. Some herbicides have been shown to leave residues that cause lepidopteran larvae to stop feeding on herbicide-exposed plants, and also some herbicides directly inhibit enzymes within the exposed insects (as discussed in Russell and Shultz 2009, and in Bohnenblust et al. 2013).

For example, glufosinate may have direct effects on lepidopteran pollinators when larvae eat glufosinate-containing pollen, nectar or leaves, either after direct over-spray or from drift. Laboratory experiments with the skipper butterfly *Calpodest ethlias* showed that larvae fed glufosinate-coated leaves were injured or killed by inhibition of glutamine synthase, at doses "comparable to the amount that might realistically be acquired by feeding on GLA [glufosinate]-treated crops." These studies were done with the active ingredient, not a full formulation, and so may have underestimated field toxicity (Kutlesa and Caveney 2001). Glufosinate is one of the herbicides that will be used with Enlist soybeans.

**Toxicity of metabolites that result from activity of novel enzymes must be assessed for non-target organisms**

When commenting on the EAs for Enlist corn and soybeans (CFS Science Soy at 84 – 94, CFS Enlist Corn II Comments at 29 - 34), CFS alerted APHIS to the need to consider potentially toxic metabolites of 2,4-D as part of its assessments, but APHIS has not done so. In fact, APHIS makes an explicit assumption that there are no differences in composition between Enlist corn and soybeans and non-2,4-D-resistant counterparts:

The APHIS PPRA did not identify any changes in DAS-40278-9 corn, DAS-68416-4 soybean, or DAS-44406-6 soybean that would directly or indirectly affect natural or biological resources. These plants are compositionally similar to other corn and soybean plants. (EIS at 119).

However, the PPRA analysis was based on compositional comparisons made in the absence of 2,4-D.

CFS reiterates that APHIS, in making a decision to approve Enlist corn and soybeans, must go beyond a description of the genotypes resulting from genetic engineering of corn and soybeans to be 2,4-D resistant, to describe and assess the PPA impacts of significant changes in the phenotypes of Enlist corn and soybeans, in environments that they are likely to be grown. Instead, APHIS has limited its assessment of important aspects of phenotypes of Enlist corn and soybeans to environments that these crops will rarely encounter – environments that are absent applications of 2,4-D.



According to 7 CFR 340.6(c), required data and information must include, among other things:

- (3) A detailed description of the **differences in genotype** between the regulated article and the nonmodified recipient organism...
- (4) A detailed **description of the phenotype** of the regulated article. Describe known and potential differences from the unmodified recipient organism that would substantiate that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it was derived, including but not limited to: Plant pest risk characteristics, disease and pest susceptibilities, **expression of the gene product, new enzymes, or changes to plant metabolism**, weediness of the regulated article, impact on the weediness of any other plant with which it can interbreed, **agricultural or cultivation practices, effects of the regulated article on nontarget organisms**, indirect plant pest effects on other agricultural products, transfer of genetic information to organisms with which it cannot interbreed, and any other information which the Administrator believes to be relevant to a determination. Any information known to the petitioner that indicates that a regulated article may pose a greater plant pest risk than the unmodified recipient organism shall also be included.

The genotype of an organism consists of its entire set of genes that contain “instructions” for making RNA and proteins that ultimately determines that organism’s characteristics. For Enlist corn and soybeans, their genotypes differ from non-engineered counterparts by the addition of DNA encoding a protein with enzymatic activity that can metabolize 2,4-D into non-phytotoxic compounds, allowing the engineered crops to withstand otherwise lethal doses of the herbicide. This transgene is *aad-1* in Enlist corn and *aad-12* in Enlist soybeans, encoding the enzyme AAD, aryloxyalkanoate dioxygenase. Other genotypic changes include sequence changes as a result of insertion of the transgene, and mutations caused by tissue culture during the engineering process. The engineered gene is embedded in the plants’ chromosomes and is passed on to all cells in the organism during development, and from one generation to the next, along with all the other corn or soybean genes.

The phenotype of an organism is “[t]he physical appearance or biochemical characteristics of an organism as a result of the interaction of its genotype and the environment” (Biology Online Dictionary 2014). For corn and soybeans, the phenotype includes size and shape, growth rate, response to environmental conditions such as day length or drought, pest and pathogen susceptibility, and other characteristics that can be observed. Phenotype also includes biochemical characteristics that are not visible to the naked eye, but can be measured with various devices, such as levels of proteins, carbohydrates, lipids, and metabolites that result from enzyme activity.

An example of the importance of metabolism as a phenotypic characterization comes from medicine. Genes for metabolizing specific drugs vary within human populations, so that the same dose of a drug may affect individuals differently, from being ineffective to causing a toxic overdose (Zanger and Schwab, 2013; Johansson and Ingelman-Sundberg, 2010). In some cases, how a person will respond can be predicted by examining the genotype, because particular enzymes encoded by specific gene variants have been shown to speed up or slow down metabolism of that drug. However, the most reliable way to tell is to measure the phenotype

directly. Physicians measure the metabolites of specific pharmaceuticals in patients after exposing them to the drug to determine the person's metabolic phenotype – how quickly they are able to down the drug – in order to personalize doses of medications to prevent overdoses and to optimize efficacy (Gumus *et al.*, 2011).

Plants with identical genotypes are likely to have different characteristics – different phenotypes – when grown in different environments. Genes have to become active in directing synthesis of RNA and proteins in order to have any effect on the characteristics of the organism; they must be “expressed” (see Alberts *et al.* 2009 for review of gene expression). Genes that are not expressed do not contribute to the phenotype of the organism. Many genes are only expressed in certain tissues and organs during development. The environment also influences how genes are expressed, and what effect the proteins made from the genes will have (Richards *et al.* 2012). For example, some genes are only turned on in the presence of external triggers, such as light or presence of a specific chemical. Some proteins produced from gene activation only function in certain conditions, as well, needing particular levels of nutrients, range of temperatures, or presence of substrates to carry out their roles.

In order to determine impacts of Enlist corn and soybeans, APHIS first must describe how Enlist corn and soybeans differ in phenotypic characteristics as a result of the specific genetic engineering events. The first step in doing so is to determine expression patterns of the transgenes, by finding out where, when, and how much of the gene products are made in the Enlist corn and soybean plants in environments in which they are likely to be grown. In this case, the engineered gene products are enzymes that break down, or metabolize, 2,4-D and some related herbicides. In its Petitions, Dow provides APHIS with some transgene expression data. They measured AAD-1 and AAD-12 protein in a few plant parts and stages of development of Enlist corn and soybeans grown with different combinations of the herbicides that the introduced enzymes allow them to withstand (see DAS Petitions, “Characterization of Introduced Proteins”).

APHIS uses Dow's description of when, where and how much of the transgenic protein is present in Enlist corn and soybean plants, along with analyses of protein sequence comparisons to known toxins and allergens, and *in vitro* studies of AAD-1 and AAD-12 protein digestion (EIS at 111), to determine whether ingestion of the transgenic proteins themselves was likely to harm non-target animals. For example, for Enlist soybeans:

DAS evaluated the potential allergenicity and toxicity of the AAD-12 protein following the weight-of-evidence approach (DAS, 2010a). The AAD-12 protein does not share any meaningful amino acid similarities with known allergens. The AAD-12 protein is degraded rapidly and completely in simulated gastric fluids, and the protein is not present in a glycosylated state (DAS, 2010a). The protein does not share any amino acid sequence similarities with known toxins (DAS, 2010a). The results presented by DAS suggest that the AAD-1 protein is unlikely to be a toxin in animal diets. Based on a review of this information and **the assumption that these studies serve as surrogates for direct testing**, APHIS has found no evidence that the presence of the *aad-12* gene or the expression of the AAD-12 protein would have any impact on animals, including animals beneficial to agriculture (USDA-APHIS, 2012a). (EIS at 111 – 112)

The assumption that Dow's *in silico* (computer simulated) and *in vitro* studies of AAD-1 and AAD-12 proteins can predict toxicity of these proteins, as they exist within Enlist corn and soybean plants, is unfounded. Proteins made in plants can have different properties than counterpart proteins in bacteria that were used in the simulated digestion studies, and computer analyses of coding sequences do not always identify toxins and allergens accurately (Freese and Schubert 2004). But the biggest problem with APHIS' assumption is that Dow's analyses are based on toxicity to mammals and, by extension, to humans; whereas the non-target organisms that could be impacted by approval span the taxonomic spectrum, from beneficial soil annelids (i.e. earthworms) to insect pollinators and endangered birds. Human and mammalian parameters of toxicity are simply not applicable over this range of organisms.

CFS stressed this point in our comments about analysis of harms to pollinators (CFS Science Corn II at 35 - 41, CFS Science Soy at 93 – 94). Composition of pollen, nectar and guttation liquid was not determined to assess differences resulting from the Enlist events, for example. The inadequacy for pollinators of toxicity assessments based on mammals was also stressed in a recent EPA white paper on pollinator risk assessments (EPA SAP 2012). Nor were impacts on honey bees studied by Dow in its field trials. Therefore, there are no relevant data for making an assessment of impacts of approval to honey bees or other pollinators.

In addition, APHIS must continue on in its analyses, past the characteristics of the novel proteins themselves, to determine how the functioning of the AAD enzymes changes the phenotypic characteristics of corn and soybean plants, and whether the changes could harm non-target species. As with the levels of AAD proteins, these phenotypic differences in metabolism should be described and assessed in the presence of the herbicides that will be used with Enlist corn and soybeans.

Dow's whole purpose in engineering corn and soybeans with these particular transgenes is to have the genes expressed throughout the plants at high enough levels that the resulting proteins will be *active* in converting 2,4-D to non-phytotoxic metabolites. The rate and extent of conversion of 2,4-D to metabolites, and thus the level of 2,4-D and metabolites, is the most relevant phenotypic difference to consider after looking at the properties of the novel protein itself, and this is not considered by APHIS in their assessments.

As CFS has noted (CFS Science Corn II at 29 – 34), CFS Science Soy at 84 – 92), Dow's studies of metabolites in Enlist corn and soybeans after applications of 2,4-D show that the activity of the AAD-1 and AAD-12 enzymes metabolizes 2,4-D into 2,4-DCP, that then is changed by other enzymes in the plant into conjugated forms of DCP (mainly DCP with specific sugars attached). In non-engineered corn and soybeans, little 2,4-DCP is produced after 2,4-D applications, nor are conjugated forms found at appreciable levels. 2,4-DCP has been shown to be toxic to some organisms, and conjugated forms have been shown to release 2,4-DCP during digestion, raising the specter that conjugated forms could be a delayed-release poison. Dow did not perform studies to test toxicity of these metabolites to non-target organisms, other than simply observing that insects were found in fields of Enlist corn and soybeans at levels comparable to non-engineered corn and soybeans (DAS Petition). These observations do not constitute an appropriate study of toxicity, nor do they address the range of organisms of interest. No

observations of any kind were made of pollinators, beneficial soil organisms, or predators of crop pests, for example. Nevertheless, APHIS accepts these observations as evidence that no harm to animals of ingesting Enlist corn and soybeans will occur (e.g., 44406-6 soybean PPRA, at 10: “Field observations of DAS-44406-6 (DAS and MS Tech 2011, section 7) revealed no negative effects on non-target organisms, suggesting that the production of the ADD-12, PAT and EPSPS proteins in the plant tissues are not toxic to organisms.”).

Therefore, to summarize, APHIS does not describe or consider important aspects of the known and potential differences in phenotypes of Enlist corn and soybeans that could harm non-target organisms, relative to the unmodified recipient organisms, in the environmental conditions that Enlist corn and soybeans are likely to encounter. APHIS only considers toxicity of the protein products of the AAD-1 and AAD-12 transgenes (the earliest phenotypic character), rather than following through to consider how these new enzymes would change plant metabolism in such a way that the plants’ phenotypes would differ in the most likely environment for Enlist crops, where 2,4-D will be present. In the likely and foreseeable presence of 2,4-D, potentially toxic metabolites accumulate in the Enlist corn and soybeans but not in the recipient organisms. APHIS does not consider impacts of these potential toxins as part of the approval process or other assessments.

**APHIS uses inappropriate and inadequate studies of nutritional value and toxicity of Enlist corn and soybeans to assess risks to threatened and endangered species, and ignores risks from herbicide applications**

**Risks to listed species known to eat corn and soybeans are not considered**

Again, APHIS relies on Dow’s presentation of “food and feed safety” of the AAD-1 and AAD-12 proteins to conclude that exposure and consumption of Enlist corn and soybeans would have no effect on threatened or endangered animal species, or those proposed for listing (Enlist corn: EIS, at 153 – 154; Enlist soybeans: EIS, at 156 – 156). As discussed above, nutritional requirements and toxicity differ between species, so that extrapolation from mammalian requirements is not valid for assessing risk to other animal taxa. For example, insects may eat nectar or pollen that was not studied for differences in nutrient composition. Birds may eat insects that fed on corn or soybean leaves, and the insects were not studied to see if they differ nutritionally. In addition, APHIS did not look at risks from potentially toxic metabolites in relevant Enlist corn- or soybean-derived materials used by endangered species that result from activity of the introduced enzymes in the presence of 2,4-D.

APHIS claims that no listed animal species use corn and soybean plants as “hosts”, without defining what is mean by host (for example, EIS at 154: “APHIS considered the possibility that DAS-40278-9 corn could serve a host plant for a threatened or endangered species. A review of the species list reveals that there are none that would use corn as a host plant.”). There may or may not be listed species that use corn and soybean plants as their main food source to complete segments of their lifecycles, but there are certainly listed animals that forage for food in corn and soybean fields.



APHIS did mention listed birds that might be found in soybean fields (EIS at 153), discounting any significant impacts based on a study showing that at least some of these birds don't consume soybeans:

Few if any TES are likely to use soybean fields because they do not provide suitable habitat. Only whooping crane (*Grus americana*), sandhill crane (*Grus canadensis pulla*), piping plover (*Charadrius melodus*), interior least tern (*Sterna antillarum*), and Sprague's pipit (*Anthus spragueii*; a candidate species) occasionally feed in farmed sites (USFWS, 2011a). These bird species may visit soybean fields during migratory periods, but would not be present during normal farming operations (Krapu *et al.*, 2004; USFWS, 2011a). In a study of soybean consumption by wildlife in Nebraska, results indicated that soybeans do not provide the high energy food source needed by cranes and waterfowl (Krapu *et al.*, 2004). (EIS p. 156)

Some listed mammals were also identified by APHIS as being found in soybean fields on occasion:

The Delmarva fox squirrel (*Sciurus niger cinereus*), which inhabits mature forests of mixed hardwoods and pines, may be found adjacent to agricultural areas of the Delmarva Peninsula (USFWS, 2011b). ... The Louisiana black bear (*Ursus americanus luteolus*), occurring in Louisiana, Mississippi, and Texas (Johnsen *et al.*, 2005), may occasionally forage on soybean; however, other crops such as corn, sugarcane, and winter wheat are preferred by the species (MSU, No Date). (EIS at 156)

APHIS fails to also consider listed species that might be found in Elist cornfields, even though in discussing soybeans APHIS admits that the Louisiana black bear prefers corn to soybeans (EIS at 156). Also, in the Nebraska study of birds in agricultural fields, cited by APHIS for lack of soybean consumption, corn is an important food source (Krapu *et al.* 2004).

Certainly, corn plants and seeds are eaten by at least one endangered migratory bird, the whooping crane (*Grus americana*), both as they forage naturally and in their "chow" when chicks are raised by conservation groups. Soybeans are also added to their chow. For example, the International Crane Foundation (ICF) answers questions about what cranes, including whooping cranes, eat, noting that they cranes eat enough newly sprouted corn and seeds to make them a nuisance in some fields (ICF 2014):

## Feeding

### Q: What do the cranes at ICF eat?

A: At ICF, cranes eat "crane chow", a special blend of soy, alfalfa, fish, and corn meal, with a special vitamin supplement. All species get the same diet, although protein content changes with the season and the bird's age. Breeding females also get calcium chips in spring to help with eggshell formation, and all the cranes get shelled corn in winter, to provide extra carbohydrates.

**Q: Do cranes cause crop damage?**

**A:** Yes, on occasion they will. In Wisconsin, cranes may cause crop damage in corn and potato fields, where the birds may feed on newly sprouted corn plants or maturing potato tubers. Members of the ICF Field Ecology Department are involved in a long-term study of crop depredation in a study area located near Briggsville, Wisconsin. ICF researchers are working with local farmers to develop a substance to put on corn kernels that will taste bad to cranes, with the hope that this will deter them from feeding in treated fields. Farmers throughout the world are faced with this challenge, and solutions developed in Wisconsin may be useful for farmers in other countries.

Whooping cranes are not numerous enough yet to cause much damage to fields, relative to other crane species, but they are found in mixed flocks with Sandhill cranes and exhibit the same feeding behavior.

The experts at Operation Migration, who guide whooping cranes in the Eastern population with ultralight aircraft on their first migration, note that the birds forage in soybean fields as well, so may be exposed to Enlist soybean residues even if they aren't eating soybean seeds (Operation Migration 2013):

**Karen Anne** April 1, 2013 1:35 pm

What do the whoopers eat when there's snow on the ground?

**Heather Ray** [from Operation Migration] April 1, 2013 4:18 pm They can and do still find a variety of foods – seeds, fruit/berries, and they travel to corn and soybean fields to consume waste grain.

The fact that whooping cranes eat young corn plants means that the birds may be present in fields shortly after over-the-top herbicide applications are made to Enlist corn. The 2,4-D residues and metabolites in newly-sprayed seedling corn have not been reported by Dow in its residue and metabolite studies, nor have Enlist corn seedlings been examined for other compositional differences, so APHIS cannot claim that food and feed studies show lack of risk to listed species.

**APHIS does not analyze risks to listed species from exposure to herbicides used with Enlist corn and soybeans**

In assessing potential effects of Enlist corn and soybeans on endangered plants, and on critical habitat that is composed of particular vegetation, APHIS does not consider impacts of herbicide use with Enlist corn and soybeans at all (EIS at 153, 156). However, in Appendix 8, APHIS provides information from EPA Environmental Fate and Effects Division showing that non-listed plants are at potential risk from direct effects of drift and runoff of 2,4-D choline use on Enlist corn and soybeans (EIS Appendix at 8-10). Some non-listed animals are also at risk from direct effects of exposure to 2,4-D choline, and "...all non-listed taxa [are identified] as potentially at indirect risks from the proposed uses of 2,4-D choline salt because of potential dependencies (e.g., food, shelter, habitat) on species that are directly affected." (EIS Appendix at 8-10)

Listed species identified as being at potential risk from 2,4-D choline applications to Enlist corn and soybeans are also being assessed by EPA (EIS Appendix at 8-10).

Enlist corn and soybeans are genetically engineered for resistance to herbicides in addition to glyphosate and 2,4-D, and use of these other herbicides with Enlist corn and soybeans must be analyzed for harm to listed species:

- Enlist corn is resistant to quizalofop in addition to 2,4-D, and APHIS provides information on EPA's screening level ecological risk assessment for listed and non-listed species for the proposed label for quizalofop in Appendix 8 (EIS Appendix at 8-18). There are possible direct effects to various animals and plants, and also the potential for habitat modifications for all listed taxa.
- Enlist soybeans are also resistant to glufosinate, and APHIS expects glufosinate to be used as it is on other glufosinate resistant soybean events (Liberty Link soybeans) (EIS Appendix at 8-20). CFS discusses potential risks to various taxa of glufosinate as it will be used with Enlist soybeans in relation to beneficial organisms, above.

APHIS cannot rely on EPA to analyze the foreseeable impacts of use of quizalofop and glufosinate on Enlist corn and soybeans, but must itself analyze impacts of these herbicides to listed species, as for use of 2,4-D with Enlist corn and soybeans.

Given this preview from EPA, it is clear that some listed species will be at risk from the approval action by APHIS of Enlist corn and soybeans, and that APHIS cannot improperly delegate responsibility for these potential harms of its action.

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# **Dow AgroSciences Petitions (09-233-01p, 09-349-01p, and 11-234-01p) for Determinations of Nonregulated Status for 2,4-D-Resistant Corn and Soybean Varieties**

## **Final Environmental Impact Statement—August 2014**

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## Executive Summary

Summarized as “Protecting American Agriculture,” the mission of the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) is “To protect the health and value of American agriculture and natural resources.”<sup>1</sup> APHIS regulates plant and animal health to achieve its mission. It integrates these regulatory functions to help ensure beneficial impacts on United States (U.S.) domestic agricultural production, commodities, trade in agricultural products, and the environment.

One function critical to the APHIS mission is preventing the introduction and distribution of plant pests. This includes the management of certain plants, animals, and microorganisms that harm plants and cause economic losses to U.S. agriculture. It extends to practices and technologies that have the potential to increase plant pest risks.

The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) received three requests (petitions) from Dow AgroSciences (DAS) seeking determinations of nonregulated status for genetically engineered (GE) plant varieties referred to as: DAS-40278-9 corn, DAS-68416-4 soybean, and DAS-44406-6 soybean (also known as Enlist™ corn and soybean). Currently, these GE plant varieties are regulated by APHIS, and Dow is seeking authorization from APHIS to grow these varieties without any APHIS regulatory oversight. Since these three GE plant varieties are currently under APHIS’ regulatory oversight, Dow must comply with a full range of safeguarding measures to ensure that these regulated GE plant varieties do not transfer or spread from their outdoor plantings while under regulation. A permit is also required to move these regulated varieties interstate. Even if APHIS grants authorization to Dow to grow these varieties, two other agencies, the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA), also provide oversight of genetically engineered plants (described below).

As described in more detail below, APHIS authority for regulation of certain GE organisms is provided in the Plant Protection Act. Once a developer of a GE plant has obtained enough information to conclude that its regulated GE plant is unlikely to cause injury, damage, or disease to plants or plant products (that is, pose a plant pest risk), the developer may petition APHIS to remove the GE plant from regulation. Usually the petition is received when the developer wishes to commercialize a specific GE plant variety. This petition is referred to as seeking “nonregulated status” or as requesting a “deregulation.” If the petition for nonregulated status is approved by APHIS, then APHIS’ permits would no longer be required to grow or ship the genetically engineered plant and its progeny throughout the U.S. and its territories. This scenario would be the case if APHIS determines that nonregulated status is appropriate for one or more of the three Dow GE varieties. If nonregulated status is granted, herbicide applications

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<sup>1</sup>For more details about the APHIS mission, visit [http://www.aphis.usda.gov/about\\_aphis/](http://www.aphis.usda.gov/about_aphis/)

to these crops would still be under EPA oversight and food safety of the crops would still be under FDA oversight as described below.

## **Regulatory Authority**

APHIS regulates certain GE organisms under authority provided in the Plant Protection Act (PPA) of 2000 as amended (7 U.S.C. §§ 7701–7772), and by APHIS’ regulations codified in Title 7, part 340 of the U.S. Code of Federal Regulations (7 CFR part 340). APHIS’ part 340 regulations govern a GE organism: if it is a plant pest (such as certain microorganisms or insects that can cause injury or damage to plants); or, if it is created using an organism that is itself a plant pest; or, if APHIS does not know or cannot determine if the GE organism is or may be a plant pest.

Any party can petition APHIS for “nonregulated status” of a GE organism (that is, to discontinue regulating a GE organism that falls under its regulations) through the procedures described in 7 CFR § 340.6. APHIS regulates such a GE organism until the agency evaluates whether the GE organism meets the PPA definition of a plant pest and concludes on the basis of scientific evidence that the GE organism is unlikely to pose a plant pest risk; that is to say that the potential for the GE organism to cause plant disease or damage is unlikely. In this case, the petitioner must provide data usually gathered through confined field tests regulated by APHIS to help inform the agency’s decision. APHIS analyzes the data from the petitioner, researches current scientific findings, and prepares a plant pest risk assessment (PPRA) that documents whether or not the GE organism is likely to cause disease or damage. If APHIS concludes that the GE organism is unlikely to pose a plant pest risk, APHIS must then issue a regulatory determination of non-regulated status, since the agency does not have regulatory authority to regulate organisms that are not plant pests. When a determination of nonregulated status has been issued, the GE organism may be introduced into the environment without APHIS’ regulatory oversight. If non-regulated status is determined for the GE corn and soybeans discussed in this EIS, Dow will be able to market the GE seeds to farmers for planting, and farmers will be able to grow, harvest, and move their crop into commerce for food and feed without any further authorization from APHIS.

Two other agencies, the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA), also provide oversight of genetically engineered plants. The relative roles of the USDA (through APHIS), FDA, and EPA are described in the “Coordinated Framework,” a 1986 policy statement from the Office of Science and Technology Policy that describes the comprehensive policy for ensuring the safety of biotechnology research and products (US-OSTP, 1986).

The FDA’s regulation of genetically modified plants is based upon its authority to regulate food safety under the Federal Food, Drug, and Cosmetic Act (FFDCA) 21 U.S.C. §§ 301 – 399. The FDA has the authority to remove adulterated food from the national food supply, which could include removing food derived from genetically modified plants.

The EPA governs the use, sale, and labeling of herbicides used on all plants pursuant to its authority under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. §§

136–136y). FIFRA governs the use, sale, and labeling of herbicides and the EPA’s actions under FIFRA directly affect the production methods used on herbicide resistant GE plants. An herbicide must first be “registered” by the EPA before it can be distributed or sold in the United States (7 U.S.C. §§ 136a(a), 136j(a)(2)(F)). The EPA registration process starts with the herbicide manufacturer providing the EPA with information about the herbicide (7 U.S.C. § 136a(c)(1)(C), (F)). The agency then evaluates any adverse effects it may have on humans and the environment. On the basis of this evaluation, the EPA then determines if it will allow the herbicide’s use on a plant, and, if so, in what quantity. The EPA sets the conditions for the herbicide’s use and places them in labeling instructions that a user must follow (*see* 7 U.S.C. § 136j(a)(2)(G)). The EPA reevaluates the herbicide every fifteen years, as part of a “re-registration process” in which the agency determines if it should continue allowing the herbicide’s use (7 U.S.C. § 136a(g)(1)(A)(iv)). The Enlist™ corn and soybean that are the subject of this EIS have been engineered to be resistant to the herbicide 2, 4-D. The EPA re-registered 2,4-D in 2005 (US-EPA, 2005b). EPA is currently reviewing the use of 2,4-D on Enlist™ corn and soybean to determine whether the herbicide would cause any unreasonable environmental risks if it were applied in accordance with its labeling instructions. The EPA does not regulate Enlist™ corn and soybean plants, because the plant itself does not produce or secrete a pesticide.

### **Purpose of Enlist™ Corn and Soybean**

Dow has developed Enlist™ GE plant varieties as alternatives to currently available GE herbicide-resistant (HR) corn and soybean varieties (see the petitions which are available on APHIS’ website [http://www.aphis.usda.gov/biotechnology/petitions\\_table\\_pending.shtml](http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml)). Many HR corn and soybean varieties have been engineered over the past 15 to 20 years. These include varieties with resistance to the herbicide glyphosate (the active ingredient in Roundup®); varieties resistant to the herbicide glufosinate (the active ingredient in the herbicide Liberty®); varieties resistant to a class of herbicides known as sulfonylureas (active ingredients in herbicides such as Glean®); varieties resistant to the synthetic auxin known as dicamba (the active ingredient in herbicides such as Banvel®), and varieties resistant to isoxaflutole and mesotrione (the active ingredient in herbicides such as Balance® and Callisto®). By far, Roundup Ready® crops have been the most widely adopted by growers. Roundup Ready® crops greatly simplified weed management for growers and reduced their weed management costs. These Roundup Ready® crops were so successful that many growers grew only Roundup Ready® crops on their farms. As another example of this success, most soybean growers could manage weeds by using glyphosate as the only herbicide, whereas three to four herbicides were previously needed.

This nearly exclusive use of glyphosate over the past fifteen years led to the selection of glyphosate-resistant (GR) weeds, weeds that could survive an application of the herbicide that once would kill earlier generations. Herbicides do not create resistant weeds, but rather, through time, individual plants may survive a treatment. In a field of weeds, individual plants vary in their genetic makeup and in their resistance to a particular herbicide. Plants that survive the herbicide treatment may produce seed, resulting in even more plants that are resistant to the herbicide. Plants that are not resistant die and do not leave off-spring. In this way, the herbicide “selects” for resistant plants and against sensitive plants and the resistant plants are disseminated

as a result of seed production and dispersal. (To read more about herbicide-resistant weeds see Appendix 6.)

Where GR weeds are widespread, the benefits of the Roundup Ready<sup>®</sup> system are diminished, and weed management is more costly. To manage GR weeds, growers have reverted to other herbicides and to mechanical cultivation practices used to manage weeds before the introduction of Roundup Ready<sup>®</sup> crops. Growers are also increasingly adopting glufosinate-resistant and other HR crops. (To read about the socioeconomic impacts of resistant weeds, see Section 5.7.1.)

The primary purpose of Enlist<sup>™</sup> corn and soybean varieties is to help growers manage GR weeds. Each of the Enlist<sup>™</sup> varieties has a trait that makes the plant resistant to the herbicide 2,4-D. 2,4-D is an active ingredient in hundreds of herbicide formulations and is commonly found in lawn care products (for example Scotts<sup>®</sup> Turf Builder<sup>®</sup> Weed and Feed). These 2,4-D products are used by homeowners and professional lawn care companies. Many lawn grasses are naturally resistant to 2,4-D. It is the third most widely used herbicide in the U.S. (glyphosate and atrazine are number one and two, respectively) and is widely available to consumers at retail outlets and home and garden centers. 2,4-D is also a relatively inexpensive herbicide. Increasingly, corn and soybean growers are using herbicide formulations that contain 2,4-D to manage GR weeds, often applying the herbicide before planting the crop. (To read more about trends in herbicide use on corn and soybean see Appendix 4.) Corn, for example, is naturally resistant to 2,4-D during certain early stages of growth. The Enlist<sup>™</sup> traits would allow growers to apply 2,4-D when the corn is older.

Enlist<sup>™</sup> corn is also resistant to, quizalofop, an herbicide found in herbicide formulations such as Assure<sup>®</sup> and Matador<sup>®</sup>. Corn is normally sensitive to quizalofop and this herbicide is sometimes used to control corn volunteers (i.e., corn that sprouts in fields now being used to grow so-called rotation crops such as soybean). Therefore, these herbicide formulations would no longer be useful for controlling volunteer Enlist<sup>™</sup> corn in soybean fields. (For more discussion of the control of corn volunteers see Plant Communities in Section 4.1.2.)

Soybean is extremely sensitive to 2,4-D, so the herbicide can only be used on soybean fields at least 30 days before planting the soybeans. In contrast, growers could apply 2,4-D to soybeans with the Enlist<sup>™</sup> trait, after the soybeans start to grow, killing the weeds but not the soybeans. Consequently, Enlist<sup>™</sup> soybean will be valuable to control the many weeds that begin to grow later in the season after the soybean has begun to grow.

In addition to resistance to 2,4-D, the commercial seed varieties of Enlist<sup>™</sup> corn are expected to have resistance to glyphosate and quizalofop and the commercial varieties of soybean are expected to have resistance to glyphosate and glufosinate. For the technical details on the creation of these three GE plants, see the petitions which are available on APHIS' website ([http://www.aphis.usda.gov/biotechnology/petitions\\_table\\_pending.shtml](http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml)).

### **Purpose and Need for Agency Action**

APHIS's regulations require that APHIS make decisions on the petitions it receives for nonregulated status. The Agency can choose to approve a petition in whole or in part, or it can



deny the petition. The decision is based on a plant pest risk assessment for the GE plants that are the subject of the petition. Plant pest risks are those risks that can cause injury, damage, or disease to plants or plant products.

The purpose of the petition process and the decisions made under the regulations is to protect plant health. Developers who can demonstrate through this process that their products do not cause plant pest risks can enter their products into commerce without restrictions after a determination of nonregulated status is made. APHIS must make a decision that is consistent with the Agency's regulatory and statutory authority.

## **Public Involvement**

In response to the receipt of the three Dow petitions, APHIS prepared preliminary PPRA's to assess the plant pest risk for each plant variety. Additionally, APHIS conducted an environmental analysis consistent with its obligations under the National Environmental Policy Act (NEPA). NEPA regulations provide that an agency shall conduct an environmental assessment (EA) to determine if an agency action will significantly affect the environment (40 CFR § 1501.4). If the agency concludes in its EA that its action will not significantly impact the environment, the agency issues a "Finding of No Significant Impact," and the agency can proceed with its proposed action without preparing an environmental impact statement (EIS). If this initial assessment finds that the agency's action may significantly affect the environment, the agency must then prepare an EIS.

APHIS examined the environmental impacts of its potential decisions for nonregulated status of the Enlist™ corn and soybean by preparing EAs for two of the three petitions: petition 09-233-01p for Enlist™ corn and petition 09-349-01p for Enlist™ soybean (this EA only considered the Enlist™ soybean variety, DAS-68416-4). Each EA included a public comment period (corn: December 27, 2011-February 27, 2012; soybean: July 13-2012-September 11, 2012). For the corn and soybean EAs, comments are accessible at <http://www.regulations.gov/#!documentDetail;D=APHIS-2010-0103-0001> and <http://www.regulations.gov/#!documentDetail;D=APHIS-2012-0019-0001>. An EA was not prepared for the Enlist™ soybean variety, DAS-44406-6, because it was submitted considerably later than the other Enlist™ corn and soybean petitions. By the time an EA would have been started, a decision had been made to prepare an EIS for all three of the Enlist™ plant varieties. As an EIS involves a more detailed environmental review, an EA was no longer necessary. APHIS has included the petition 11-234-01p corresponding to the Enlist™ soybean DAS-44406-6 in this EIS analysis because this soybean variety exhibits the same or similar HR traits as DAS-68416-4 soybean, so it presents similar environmental issues. APHIS also had a public comment period on the Notice of Intent (NOI) to prepare this EIS (May 16, 2013-July 17, 2013). APHIS received 41 comments on the NOI (See Appendix 2).

The draft EIS was available for public comment from January 10, 2014-March 11, 2014. APHIS held a public meeting on the draft EIS attended by 110 participants. Twenty four participants made comments, with 9 opposed and 15 in favor of deregulation (APHIS-2013-0044). APHIS received 10,147 submissions on the draft EIS docket (APHIS 2013-0042). Of these 8940 opposed and 1082 supported the use of Enlist™ corn and soybean. The remaining 125 comments

consisted of submission of attachments, requests for extensions, or were submissions to the wrong docket. The comment summary and responses to the draft EIS are included in Appendix 9.

During the comment periods, the public identified three main issues. Following is a brief representation of those issues with a summary of our responses:

- 1) The concern that natural and biological resources would be adversely impacted by 2,4-D in anticipation of increased amounts of this herbicide being used on Enlist™ corn and soybean should these products be deregulated.

Although many commenters raised the issue about the potential for adverse impacts to the environment from the expected increased use of 2,4-D, these direct and indirect impacts are outside the scope of this EIS because the authority to regulate the impacts of herbicide use resides with the EPA under FIFRA. The EPA has conducted independent assessments of direct and indirect effects associated with the use of 2,4-D on Enlist™ corn and soybean (US-EPA, 2013a; b; c) and is making an independent action to determine whether to approve registration of Enlist Duo™ (US-EPA, 2014). USDA's authority comes from the Plant Protection Act, which limits APHIS authority to the regulation of plant pests and noxious weeds only. The three petitions submitted by Dow are evaluated pursuant to Part 340. Under APHIS's Part 340 regulations, APHIS can only consider plant pest risks when making a determination of nonregulated status. APHIS has no authority to regulate herbicide use. The EPA's registration process under FIFRA ensures that pesticides will be properly labeled and that, if used in accordance with label specifications, pesticides will not cause unreasonable harm to humans and the environment. Thus, the EPA has the authority to regulate the effects of herbicide use on humans and the environment. The EPA's regulatory responsibilities are more fully discussed in Section 1.4.2. The risk assessments used by the EPA are explained in Section 5.4.

- 2) The need by growers for Enlist™ corn and soybean to help them manage GR weeds already present on many farms across the country.

Growers are changing management practices to manage GR weeds including increasing tillage, hand weeding, and use of different herbicide chemistries. APHIS considers the impacts of GR weeds in the No Action Alternative (Section 4.1.1 Herbicides). Enlist™ crops will allow the post-emergent use of 2,4-D for weed control. In this way, they are expected to better enable growers to manage GR weeds. APHIS considers the potential for its decision on these petitions, combined with EPA's decision on the labeling of the Enlist Duo™ herbicide (a combination of 2,4-D and glyphosate) to influence management practices and to control GR weeds in Chapter 5.

- 3) The concern that increased use of 2,4-D on Enlist™ corn and soybean would hasten the selection of 2,4-D-resistant weeds.

APHIS has identified the possible selection of HR weeds resulting from the change in management practices associated with the adoption of Enlist™ corn and soybean as a potentially significant environmental impact. This impact is a cumulative impact because it would only result from the combined action of USDA on the subject petitions and of the

EPA's action to register 2,4-D for use on Enlist™ corn and soybean. If 2,4-D-resistant weeds were to be selected as a result of these combined actions, growers who rely on 2,4-D for effective and inexpensive weed control are likely to experience increased socioeconomic impacts from more costly and restrictive weed control alternatives.

Because of the likely adverse socioeconomic impacts that would result in the event that 2,4-D-resistant weeds would be selected from the expected increased 2,4-D use on Enlist™ crops, APHIS believed these impacts may be significant. Therefore APHIS decided, for the three Enlist™ varieties that are the subject of this EIS, to exercise its discretion to prepare an EIS to further analyze the potential for selection of 2,4-D-resistant weeds and other potential impacts that may occur from making determinations of nonregulated status for these varieties. This EIS limits its analysis of herbicide use to the cumulative impacts that occur from the selection of HR weeds and the changes in management practices that result so as not to duplicate the analysis independently conducted by the EPA (US-EPA, 2013a; b; c) (US-EPA, 2014)

### **Alternatives Analyzed**

In this EIS, APHIS considers four alternatives regarding the possible deregulation of these three GE organisms. The four alternatives are: (1) No Action; (2) approve the petitions for nonregulated status of Enlist™ corn and soybean; (3) approve the petition for nonregulated status of Enlist™ corn only; and (4) approve both petitions for nonregulated status of Enlist soybean™ only.

#### **Alternative 1: No Action Alternative—Continuation as Regulated Articles**

Under the No Action Alternative, APHIS would deny the three petitions and these GE plant varieties would continue to be regulated by APHIS. Permits issued or notifications acknowledged by APHIS would still be required for the introduction of Enlist™ corn and soybean, and measures to ensure physical and reproductive confinement would continue to be implemented. This Alternative is not the Preferred Alternative because APHIS has concluded in its PPRAs that Enlist™ corn and soybean are unlikely to pose plant pest risks (USDA-APHIS, 2010b; 2012a; b). Choosing this alternative would not satisfy the purpose and need of making the required regulatory determination that is consistent with the PPA and 7 CFR part 340.6.

#### **Alternative 2: Determination of Nonregulated Status of DAS-40278-9 Corn, DAS-68416-4 Soybean, and DAS 44406-6 Soybean (Preferred Alternative)**

Pursuant to its PPA authority, as implemented in 7 CFR part 340, APHIS must respond to a petition to reclassify a regulated article as not subject to regulation under the plant pest provisions of the PPA. Under this alternative, Enlist™ corn and soybean and their progeny would no longer be subject to APHIS biotechnology regulations (7 CFR part 340). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of these varieties. This alternative meets the purpose and need to respond appropriately to the petitions (DAS, 2010a; DAS, 2010b; DAS, 2011) for nonregulated status, the requirements in 7 CFR part 340, and the Agency's regulatory authority under the plant pest provisions of the PPA, because these varieties are unlikely to pose plant pest risks as evaluated in the PPRA (USDA-APHIS, 2010b; 2012a; b). Therefore, this is the Preferred Alternative because approving the

petitions for nonregulated status for all three varieties is consistent with the plant pest provisions of the PPA and the regulations codified in 7 CFR part 340.

### **Alternative 3: Approve the Petition for a Determination of Nonregulated Status of DAS 40278-9 Corn Only**

Under this alternative, only Enlist™ corn and progeny derived from its cultivation would no longer be subject to the PPA regulations. Permits issued or notifications acknowledged by APHIS would no longer be required for this corn and its progeny. This alternative meets the purpose and need to respond appropriately to the petition for nonregulated status for Enlist™ corn, the requirements in 7 CFR part 340 and the Agency's regulatory authority under the plant pest provisions of the PPA, because it is unlikely to pose a plant pest risk evaluated in the PPRA (USDA-APHIS, 2010b; 2012a; b). Therefore, approving the petition for a determination of nonregulated status for Enlist™ corn is consistent with the plant pest provisions of the PPA and the regulations codified in 7 CFR part 340. However because APHIS has concluded in its PPRAs that the two Enlist™ soybean varieties are unlikely to pose plant pest risks (USDA-APHIS, 2010b; 2012a; b), choosing this alternative would not satisfy the purpose and need of making the required regulatory determination that is consistent with the PPA and 7 CFR part 340.6.

### **Alternative 4: Approve the Petition for a Determination of Nonregulated Status of DAS-68416-4 Soybean and DAS-44406-6 Soybean Only**

Under this alternative, only the two Enlist™ soybean events (an event is a plant line produced from the insertion of a specific DNA into a plant species) and progeny derived from their cultivation would no longer be subject to the PPA regulations. Permits issued or notifications acknowledged by APHIS would no longer be required for their introduction and progeny derived from them. This alternative meets the purpose and need to respond appropriately to the petitions (DAS, 2010b; DAS, 2011) for nonregulated status for the two Enlist™ soybean varieties, the requirements in 7 CFR part 340 and the Agency's regulatory authority under the plant pest provisions of the PPA, because it is unlikely that they pose a plant pest risk evaluated in the PPRA (USDA-APHIS, 2010b; 2012a; b). Therefore, the Agency's deregulation of the two Enlist™ soybean varieties is consistent with the plant pest provisions of the PPA and the regulations codified in 7 CFR part 340. However, because APHIS has concluded in its PPRAs that Enlist™ corn is unlikely to pose plant pest risks (USDA-APHIS, 2010b; 2012a; b), choosing this alternative would not satisfy the purpose and need of making the required regulatory determination that is consistent with the PPA and 7 CFR part 340.6.

### **Affected Environment**

In order to determine the extent of the potential environmental impacts that could result from APHIS' decision whether to grant nonregulated status, APHIS used information provided by the National Agricultural Statistics Service (USDA-NASS) to identify those regions of the country where corn and soybeans are grown. To describe the ecological features of corn and soybean regions, APHIS compared these growing areas to maps that group regions having similar ecological attributes such as soil, landform, or major vegetation types (CEC 2009). These regions are termed ecoregions and are identified as Regions A through M in this EIS (see section 3.1.3).



To identify the types of land cover and crops grown in each region, APHIS analyzed information in the USDA-NASS online tool, “Cropscape” an information source that compiles these data from satellite imagery.

### **Potential Environmental Consequences of Alternatives**

Environmental issues are assessed individually in Chapter 4 (Potential Environmental Consequences). As stated previously, APHIS has regulatory authority over the Enlist™ corn and soybean plants, and the EPA has regulatory authority over the Enlist™ Duo herbicide (a premix of 2,4-D and glyphosate). The scope of this EIS covers the direct and indirect impacts that would result from the cultivation and use of the plant. The EPA, in its registration process, is considering any direct and indirect impacts from the use of the herbicide on Enlist™ plants. The USDA is relying on the EPA’s authoritative assessments and will not duplicate the assessment prepared by the EPA. USDA also considers in this EIS (Chapter 5), cumulative impacts that result in the event that USDA approves the petitions for non-regulated status to Enlist™ corn and soybean and the EPA registers the use of herbicides on these crops.

APHIS determined that Enlist™ corn and soybean varieties would not result in an increase in acres in areas already in corn and soybean production. In addition, APHIS determined that there were no direct or indirect impacts on the environment from the cultivation of Enlist™ corn and soybean plants themselves, because these GE varieties are not agronomically different from non-GE corn and soybean plants or other GE corn or soybean plants that are no longer regulated by the Agency. These three GE plant varieties do not affect natural (e.g., soil, water, air) or biological (e.g., animal, insect, plant) resources directly. Rather, the management practices (e.g., pesticide applications and tillage practices) associated with their use could impact natural and biological resources. For example, herbicide applications can lead to the selection of weeds resistant to that herbicide, and tillage can adversely affect soil, water, and air quality and increase greenhouse gas emissions. While Enlist™ corn and soybean can resist damage from the application of the Enlist Duo™ herbicide, APHIS’ selection of a particular alternative does not in itself allow the use of Enlist Duo™ herbicide on Enlist™ corn and soybean plant varieties. The EPA regulates the use of herbicides under FIFRA and is making a separate decision which may or may not allow use of Enlist Duo™ herbicide on these plants.

In Chapter 5, the environmental analysis considers potential cumulative impacts, including how herbicide use may change if the requested EPA actions are approved in conjunction with those of APHIS. APHIS approval of the three petitions for nonregulated status for Enlist™ corn and soybean and the independent decision by EPA to register Enlist Duo™ herbicide for use on these GE plant varieties is reasonably foreseeable. This herbicide product contains a unique formulation of 2,4-D mixed with glyphosate devised to reduce the off-target movement of 2,4-D. Its use is required by a Stewardship Agreement for anyone planting Enlist™ corn and soybean. For more on off-target movement see Appendix 7. One farmer group, the Save Our Crops Coalition, consisting of growers who raise 2,4-D sensitive crops, was initially opposed to Enlist™ crops due to concerns about off-target movement from the application of 2,4-D. However, their position changed based on discussions between the Save our Crops Coalition and Dow. As a result of those discussions, Dow committed to foster stewardship practices by Enlist™ technology users and amended its pending label submitted to the EPA to include further

mitigation language (APHIS-2013-0042-7255 at regulations.gov). The mitigation language aimed at drift reduction includes a requirement of Enlist™ technology users to use only Enlist Duo™, a low volatility formulation, a specific nozzle type and maximum wind speed for application, applicator reporting requirements, and a 30-foot buffer zone from sensitive areas on areas to be sprayed.

As part of the cumulative impacts analysis, APHIS analyzed trends in 2,4-D use and, with information supplied by Dow, predicted 2,4-D use in the future under each of the Alternatives (see Appendix 4, Table 4-12). Based on the existing trend of increased use of 2,4-D over the last decade (i.e., without these 2,4-D-resistant crops), APHIS projects that 2,4-D use on crops will increase by nearly 75 percent by 2020 under the No Action Alternative. If EPA registers Enlist Duo™ herbicide for Enlist™ corn and soybean and APHIS adopts the Preferred Alternative, APHIS expects that 2,4-D use will further increase on crops by 200 to 600 percent by 2020 (depending on the assumptions made) relative to current use.

Deregulation of Enlist™ crops and approval of use of 2,4-D on those crops will cause growers to change management practices; namely 2,4-D use is expected to increase beyond the increase expected without these crops. Furthermore, 2,4-D use is expected to be used over a wider part of the growing season. The change in management practices expected under the Preferred Alternative is expected to increase the pressure for selection of 2,4-D-resistant weeds. Growers themselves can influence this selection pressure by the management practices they choose. Some examples of the practices that can be followed to reduce or delay the selection of HR weeds include, rotating crops, rotating types of herbicides, using cover crops, scouting for weeds, and using mechanical tillage to prevent weeds from flowering (see Section 5.7.2 for additional discussion). Societies such as the Weed Science Society of America (WSSA), university extension agents, and industry, have made a concerted effort to increase grower awareness of best management practices for HR weeds (see for example APHIS-2013-0042-1911 and -6165). The extent to which growers will adopt best management practices is unknown and, therefore, it is difficult to accurately predict when and the extent to which 2,4-D-resistant weeds will become a problem.

As noted above, 2,4-D is already the third most widely used herbicide in the United States. Among agricultural uses, 2,4-D is widely used for weed control on small grains (wheat, barley, oats, and sorghum) and orchards (see Appendix 4, Table 4-7 for a more complete list). If 2,4-D resistant weeds become more prevalent as a result of its use on Enlist™ corn and soybean, growers of these other crops that rely on 2,4-D for weed control may need to modify management practices to control weeds that become resistant to 2,4-D. The management changes would increase the complexity and cost of weed management programs for these growers. Growers most likely to be affected include those who grow small grains (See Chapter 5 for additional discussion). To identify which areas are most likely to experience cumulative impacts, APHIS identified areas where corn or soybean and small grains are grown in proximity and represent greater than 20 percent of cropland (see Chapter 5, Table 10). The areas identified include Ecoregion D (coastal southeast), Ecoregion F (Louisiana, Mississippi, Arkansas, Tennessee, Missouri); Ecoregion H (Western North Dakota and South Dakota); Ecoregion I (western Kansas, Nebraska, eastern Wyoming, eastern Colorado, and parts of western Texas and

western Oklahoma); and Ecoregion J (central Texas). Because the use of Enlist™ corn and soybean does not require a single specific set of agronomic practices, the magnitude of the impacts discussed depends on the adoption rates of various practices by growers. While the selection pressure for 2,4-D-resistant weeds is expected to be greater under the Preferred Alternative, the selection pressure for GR weeds is expected to be greater under the No Action Alternative. This is because the Enlist™ cropping system decreases grower reliance on glyphosate by including an additional type of herbicide in the weed management system.

The continued emergence of GR weeds under the No Action Alternative will itself call for modification of crop management practices to address these weeds. Growers are expected to become less reliant on glyphosate for the control of weeds it is no longer effective in controlling. Growers will likely continue to use the herbicide because it is still effective on hundreds of weed species. However, farmers are expected to use additional chemical and non-chemical methods to control the GR weeds, too. Changes in management practices are expected to include more use of non-glyphosate herbicides and adjustments to crop rotation and tillage practices (Owen et al., 2011b). Herbicides that kill weeds by mechanisms (referred to as sites of action; see Appendix 3) different than glyphosate are expected to increase as growers face the need to manage GR weeds (see Figures 4.1 and 4.3 in Appendix 4). A site of action refers to the biochemical reaction that is affected by the herbicide. For example, glyphosate and glufosinate inhibit distinct reactions and therefore have different sites of action. 2,4-D affects yet another site of action. Current trends indicate that herbicides representing at least six sites of action are increasing in use and are expected to continue to increase under the No Action Alternative.

Selection of GR weeds is expected to continue where glyphosate is used. Areas where GR weeds are expected to remain a serious concern include the Southeast, Great Plains and Northern Crescent regions, where such weeds have already become widely prevalent. Furthermore, because other non-glyphosate herbicides will still be used to manage GR weeds, weeds resistant to non-glyphosate herbicides will continue to be selected. For many of the non-glyphosate herbicides, resistant weeds have already been selected and are widely prevalent (see Appendix 6 for more details). As a result, herbicide options for weed management may become less attractive under the No Action Alternative and growers may be forced to return to more aggressive tillage systems to maintain soybean yields (Conley, 2013).

Under the Preferred Alternative, 2,4-D use is expected to increase relative to the No Action Alternative, if EPA approves the amended use of 2,4-D on these crops. However, increases in other herbicide sites of action under the Preferred Alternative are expected to be less than under the No Action Alternative because the Enlist Duo™ herbicide is expected to be preferentially adopted if approved for use on these crops by EPA. The availability of inexpensive and effective herbicides in Enlist Duo™ combined with Enlist™ corn and soybean may delay the adoption of non-chemical management strategies under the Preferred Alternative. Fewer growers would be expected to adopt aggressive tillage when herbicides remain effective for weed control. Selection of weeds resistant to glyphosate and non-glyphosate herbicides will still occur under the Preferred Alternative. The selection pressure for HR weeds under the Preferred Alternative relative to the No Action Alternative will depend on the management practices employed under

each alternative and cannot be predicted. More diversified weed management practices will result in less selective pressure for resistance to any given herbicide or management technique.

Under the No Action Alternative, natural resources are expected to be negatively impacted by a return to more aggressive tillage practices. If conventional tillage increases to control glyphosate- and other herbicide-resistant weeds, there may be an impact on soil quality from increased erosion; on air quality from increased air particulates and increased emission due to more use of farm equipment; on water quality from increased sedimentation; on climate change from increased release of greenhouse gases from burning additional fossil fuels and soil disruption that releases sequestered carbon; and on biodiversity from habitat loss. The total acreage that may be impacted by such an increase in tillage would be based on the extent of resistant weeds present in a field and the weed management strategy chosen by a grower. Adoption of Enlist<sup>TM</sup> soybean can provide growers with an alternative herbicide to glyphosate and glufosinate and could provide growers with an alternative to intensive tillage practices that may be used to manage HR weeds. However, the eventual occurrence of weeds resistant to glyphosate, 2,4-D and glufosinate will over time limit the use of Enlist<sup>TM</sup> crops and any benefit to natural resources that may arise. The magnitude of the benefit or the loss of the benefit is uncertain because decisions on crop production management are made by individual growers.



## Monarch Butterflies and Agriculture

John M. Pleasants

More than a decade ago, agricultural fields in the U.S. Upper Midwest accounted for a high proportion of milkweeds and a large fraction of new monarchs produced during the summer months. During the past 15 years, two types of genetically modified (GM) crop plants, Bt corn and glyphosate-tolerant corn and soybeans, have rapidly dominated agricultural acreage in the Midwest. Initial concerns about the consumption of Bt corn pollen by monarchs were laid to rest but then replaced by newer concerns about glyphosate-tolerant crops. The use of glyphosate has virtually eliminated milkweeds from agricultural fields. From 1999 to 2012, the amount of milkweed on the Midwest landscape declined by 64% and Midwest monarch production declined by 88%. Over the same period, the number of overwintering monarchs declined by 72%. Milkweeds on Conservation Reserve Program land have now become an important resource for monarchs. I show that herbicide kills monarch eggs and larvae by defoliating plants. I also examine several reasons for the observed high density of monarch eggs on agricultural milkweeds and estimate the potential milkweed resource in presettlement times.

### INTRODUCTION

The presence of milkweeds (*Asclepias syriaca*) in agricultural fields has concerned farmers and weed scientists because of their impact on crop yield (Bhowmik 1994). What was not appreciated until recently was the importance of milkweeds in agricultural fields for monarchs. This realization resulted from several studies addressing the question of whether Bt corn poses a threat to monarchs. Hartzler and Buhler (2000) measured milkweed densities in different habitats in Iowa in 1999. Oberhauser et al. (2001) and Pleasants and Oberhauser (2012) used these densities and the amount of area occupied by different habitats to estimate that in 1999, approximately half the milkweeds in the Upper Midwest were in agricultural fields. Additionally, they found a higher per-plant density of monarch eggs on milkweeds in agricultural fields than on milkweeds in nonagricultural habitats, and they estimated that about 80% of all monarchs in the Midwest fed as larvae on milkweeds in agricultural fields. Because approximately half of all monarchs that overwin-

tered in Mexico in the late 1990s came from the corn belt region of the U.S. Midwest (Wassenaar and Hobson 1998), it is clear that milkweeds in agricultural fields are important for overall monarch production; in fact, over the last decade, the estimated number of monarchs produced in the Midwest correlated positively with the number of monarchs in the overwintering colonies in Mexico (Pleasants and Oberhauser 2012).

In 1996, two genetically modified (GM) crop plants with the potential to affect monarchs became available. One of these, Bt corn, contains a bacterial gene that encodes a crystal toxin protein; when incorporated into plant tissues, this protein inhibits feeding by lepidopteran larvae and targets the European corn borer in particular. The second GM crop, glyphosate-tolerant (brand name Roundup Ready) soybeans, contains a gene from a bacterium that confers tolerance to the herbicide glyphosate. Corn varieties with glyphosate tolerance have also been developed, and this trait is sometime stacked (combined into the same plant line) with the Bt trait.

### GM crops and monarchs: Bt corn

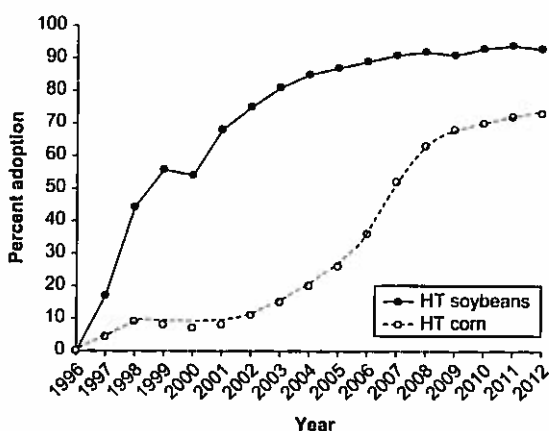
In 1999, a laboratory study raised concerns about the potential negative effect of Bt corn pollen blown onto milkweed leaves and consumed by monarch larvae ( Losey et al. 1999). At that time, 26% of corn acres were planted with Bt corn (USDA, Economic Research Service 2012). Subsequent field studies focused on the temporal and spatial overlap between monarch larvae and Bt corn pollen (Oberhauser et al. 2001), the amounts of Bt pollen to which monarchs might be exposed (Pleasants et al. 2001), and the sensitivity of the larvae to different amounts of Bt corn pollen on milkweed leaves both in the lab (Hellmich et al. 2001) and in the field (Stanley-Horn et al. 2001). Because corn pollen does not travel very far from fields (Pleasants et al. 2001; Kawashima et al. 2004), the primary danger of Bt corn pollen would be to monarch larvae feeding on milkweeds in or immediately adjacent to cornfields. Sears et al. (2001) combined all risk factors from these studies and concluded that the risk to monarchs was very small, primarily because the chance of encountering a pollen density on milkweed leaves that would reduce growth and survival was very low. In a follow-up study, Dively et al. (2004) examined monarch larvae exposed to naturally occurring Bt corn pollen in the field; they estimated that Bt pollen could result in 0.6% mortality among the monarch population as a whole, a result that could mean several hundred thousand negatively affected individuals, assuming a starting population in excess of 100 million (Garcia-Serrano et al. 2004). Their conclusion was based on a lower percentage of Bt corn plantings than exists today, suggesting that the risk today might be greater, but it was also calculated on the basis of the higher density of milkweed in agricultural fields in about 2000. The amount of milkweed in agricultural fields has been drastically reduced since that time by glyphosate herbicide (see below), eliminating concerns about Bt corn pollen.

### GM crops and monarchs: Glyphosate-tolerant corn and soybeans

Weed control practices in agricultural fields that affect milkweeds will likely affect monarchs. Historically, weed control was accomplished primarily by cultivation or tillage, but since the

1940s the application of herbicides has become an important component. Traditional herbicide use (prior to crop emergence) produced only moderate control of milkweed, such that in the 1970s and 1980s, milkweed infestation in agricultural fields was viewed to be increasing, with 10.5 million ha infested in the north-central states (Martin and Burnside 1980). The more recent adoption of no-till farming practices has made milkweed even more difficult to control (Buhler et al. 1994; Yenish et al. 1997).

Glyphosate is a potent herbicide to which milkweeds are susceptible (Bhowmik 1994; Pline et al. 2000), but because it has a detrimental effect on conventional crop plants, it could not be applied after crop emergence until the development of GM glyphosate-tolerant crops. Glyphosate-tolerant (Roundup Ready) soybeans were introduced in 1996 and had reached a 93% adoption level by 2012. Corn with the glyphosate-tolerant trait was introduced in 1998 and had reached a 73% adoption level by 2012 (Figure 14.1) (USDA, Economic Research Service 2012). Glyphosate use in soybeans went from 2.2 million kg in 1994 to 41.7 million kg in 2006 (the last year for which data are available and when adoption of glyphosate-tolerant soybeans was 89%), and glyphosate use in corn went from 2.0 million kg in 2000 to 28.5 million kg in 2010 when the adoption level was 70% (USDA, National Agricultural Statistics Service 2011).



**Figure 14.1.** Percent adoption of corn and soybeans with the herbicide (glyphosate) tolerant (HT) trait since introduction, based on data from USDA, ERS (2012). Some varieties of HT corn also have the Bt trait (stacked) while some do not.

## EFFECT OF HERBICIDE USE ON MILKWEED PLANTS

I began monitoring plots of milkweeds in six agricultural fields, between 10 and 51 ha in size, near Ames, Iowa, in 2000 and continued monitoring those plots for several years, using GPS to relocate them (see Pleasants and Oberhauser 2012 for details). This period covered the transition from nonglyphosate herbicide use to glyphosate herbicide use in conjunction with increased adoption of Roundup Ready soybeans, and later Roundup Ready corn, and thus provided an opportunity to compare the response of milkweeds to these two weed control treatments. From 2000 to 2003, I monitored each of the sites weekly for monarch activity during the study periods. In each plot (of approximately 20 m<sup>2</sup> in size), every milkweed stem was counted and inspected for monarch eggs and larvae, which were identified to instar. From 2004 to 2009, monarch activity was not monitored but the number of milkweed stems was counted once each year in early August.

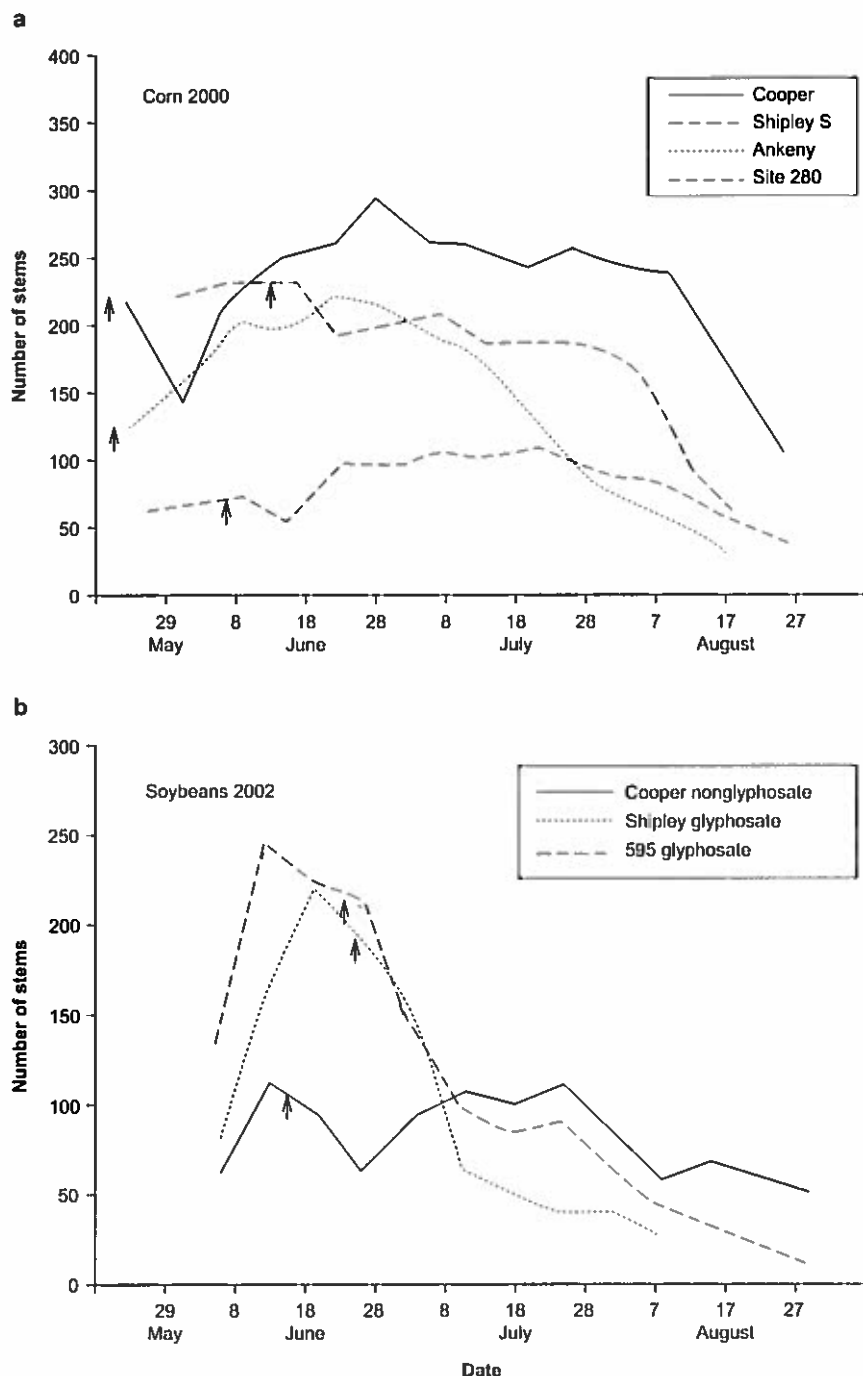
In general, whether nonglyphosate or glyphosate herbicide was used, the leaves on treated milkweed plants turned yellow within a few days of application, and then often turned brown, dried up, and fell off within a week. When nonglyphosate herbicide was used, some plants did not recover and the entire stem turned brown, but many plants sprouted new branches from leaf axils, and they appeared fully recovered in 2–3 weeks. When glyphosate herbicide was used, most plants were killed; those that survived had little if any resprouting from leaf axils.

For cornfields, the effect of nonglyphosate herbicide application on milkweed plants typically involved a reduction in the number of stems 1–2 weeks after herbicide application, followed by an increase in stems over the next 1–2 weeks to the same level or greater than just before application (Figure 14.2a). The reduction in stem number was due to stem death or apparent death, and the subsequent increase was due to stems that recovered and produced large side branches that were counted as new stems, along with the appearance of new stems by vegetative reproduction. The effect of glyphosate herbicide use in cornfields could not be evaluated because this herbicide was not applied in the cornfields examined here. In soybean fields treated with nonglyphosate herbicide, the milkweeds recovered, as seen with corn, but in fields treated with

glyphosate there was no recovery and only a small number of stems survived (Figure 14.2b).

Besides exploring the effect of herbicide use within a season on the number of milkweed stems, I also examined between-year changes in number of milkweed stems. No seedling recruitment occurred in any of the fields I examined, such that between-year changes were due to stems arising from previously existing plants. I compared the number of milkweed stems in each field near the end of one season (first week in August) with the number of stems at the same time the next year after an intervening herbicide application (Table 14.1). To examine the effect of different herbicide treatments, I used a standard least-squares linear model with treatment ("type of crop (corn or soybean)" and "herbicide" combination) and "year" as main effects, and "site" and "site by plot" as random effects. The treatment effect was significant, but the year effect was not, so the model was rerun with the treatment effect only. The treatment effect (crop and herbicide combination) was statistically significant ( $F = 28.4$ ,  $df = 3,14$ ,  $P < 0.001$ ) and all four treatments were different from each other (Tukey HSD at 0.05 level). For soybeans, the greatest decline in milkweed numbers occurred in fields sprayed with glyphosate, whereas milkweed numbers declined proportionately less in fields sprayed with nonglyphosate herbicide (Table 14.1). For cornfields sprayed with nonglyphosate herbicide, the decline in milkweed was quite low, and in the two fields where corn was planted in consecutive years, milkweed numbers actually increased (Table 14.1). For comparison, the number of milkweed stems in nonagricultural plots did not change appreciably from one year to the next (Pleasants unpublished data).

Based on these results, we can speculate about milkweed populations in agricultural fields prior to glyphosate use. The data are lacking to consider how tillage alone has affected milkweed populations, but for the majority of fields that formerly used nonglyphosate herbicide, we would expect a moderate negative effect on milkweed populations, with a greater negative effect in soybean fields than in cornfields. With corn-soybean rotation, the corn year would have allowed the milkweed population to decline less rapidly; however, with the introduction of glyphosate-tolerant soybeans and the consequent use of glyphosate herbicide, the decline of milkweeds in soybean fields would have accelerated.



**Figure 14.2.** Milkweed stem numbers in fields before and after herbicide application. Dates when herbicide was applied to each site are indicated by arrows. (a) Change in the number of milkweed stems in cornfields at 4 sites in 2000. All sites were treated with a nonglyphosate herbicide. The Ankeny site showed no reduction in numbers after application, probably because the application was ineffective for unknown reasons. (b) Change in the number of milkweed stems in soybean fields at 3 sites in 2002. Two fields, Shipley North and Site 595, were sprayed with glyphosate herbicide whereas the Cooper site was sprayed with a nonglyphosate herbicide. Cases where it appears that milkweed numbers were declining before herbicide application are an artifact of application occurring between weekly sampling intervals.



Table 14.1. Percent change in number of milkweed stems for type of crop field and type of herbicide used

Site and year	Nonglyphosate		Glyphosate	
	Corn	Corn* Beans	Beans	
Shipley N.				
2001	-40.1			
2002	-37.3			-90.0
2003		25.0		-74.7
Site 595				
2001	-16.4			
2002				-85.1
Cooper's				
2001	-14.3		-41.8	
2002	-9.4		-67.8	
2003	-17.3		-22.2	
2004	9.5		-40.3	
2005	9.3	43.5		
Airport Rd.				
2009				-87.7
Average	-14.5	34.3	-43.0	-84.4

\* Field also in corn the previous year, all other fields in crop rotation

After Roundup Ready corn was introduced, every year would have produced a significant decline in milkweeds, resulting in a precipitous decline in milkweed numbers. All the plots I examined beginning in 2000, comprising a total of about 1000 stems, were devoid of milkweeds by 2008 after experiencing a combination of glyphosate and nonglyphosate use over the years. A similar loss of milkweeds was noted in Minnesota fields (Oberhauser unpublished data).

## EFFECT OF HERBICIDE USE ON MONARCHS

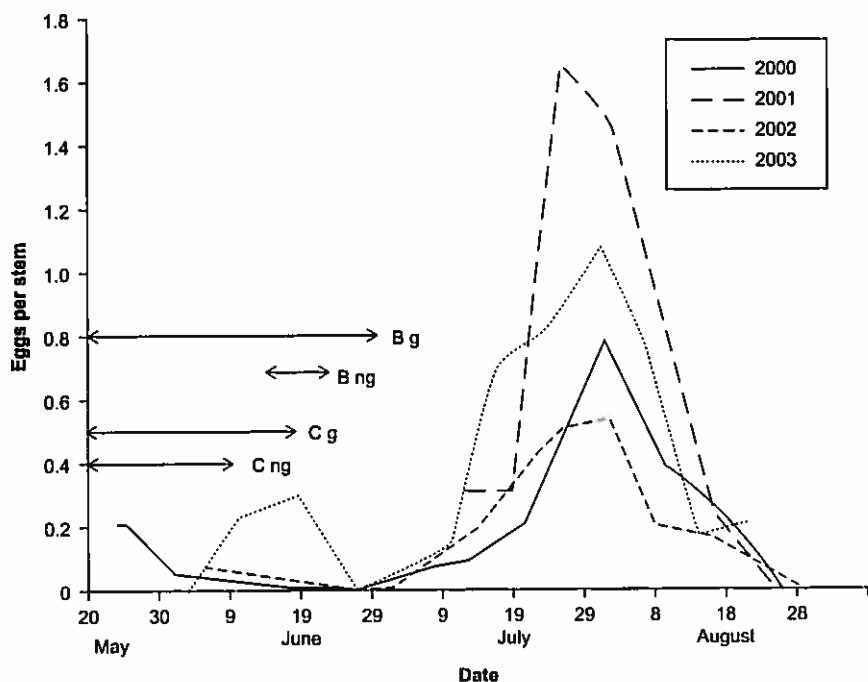
Herbicide application, either glyphosate or nonglyphosate, can have two potential effects on monarch populations. First, monarch eggs on milkweed plants at the time of herbicide application will die if defoliation occurs, and larvae will die if they are unable to move to unaffected plants. Second, when milkweeds disappear because they are killed by herbicide, it reduces the milkweed resource available to monarchs, thus potentially reducing the size of the monarch population that can be supported.

Figure 14.3 shows monarch population egg phenology (average eggs per milkweed stem in corn and soybean fields) over four seasons at study sites near Ames, Iowa. The shapes of the phenology curves for larvae per stem are very similar, although the position is shifted to the right. The phenology curves show

the two main generations of monarchs that occur in Iowa and the Midwest in general, with one generation of eggs laid in early summer (May–June) and a second (and possibly third overlapping) generation of eggs laid in July and August (Figure 14.3). In the cornfields in my study, nonglyphosate herbicide was applied from 21 May to 8 June (C ng in Figure 14.3), coinciding with first-generation eggs and larvae. Today, the majority of corn is glyphosate-tolerant. The time frame for the first application of glyphosate herbicide in glyphosate-tolerant cornfields is from 5 May to 30 May and a second application is often made 14–21 days later, as early as 19 May and as late as 20 June (C g in Figure 14.3) (ISU Weed Specialist Micheal Owen, pers. comm.). This coincides with the first monarch generation. Thus, both nonglyphosate and glyphosate herbicide application in corn appear to closely overlap the first generation of eggs and larvae and could result in monarch mortality.

In the soybean fields examined here, nonglyphosate herbicide was applied between 15 June and 23 June (B ng in Figure 14.3). Very few eggs or larvae were present at this time in three of the four years, but first-generation eggs and larvae were still present in 2003. Glyphosate herbicide was applied in late June, a period of low numbers of eggs and larvae at all sites. However, the potential time frame for the application of glyphosate herbicide to soybeans is 20 May to 10 June, with a second application sometimes two or three weeks later, as early as 3 June and as late as 1 July (B g in Figure 14.3) (Micheal Owen, pers. comm.). Thus, as in corn, the timing of glyphosate herbicide application potentially overlaps the first generation of monarchs, resulting in mortality.

I could directly document monarch deaths resulting from the defoliation caused by herbicide in 2000, 2002, and 2003, when I monitored monarch activity. I measured the survival of 13 cohorts of eggs that were present just before herbicide application based on the number of second-instar (L2) larvae present in the next census following herbicide treatment. I used cohorts in fields in which either nonglyphosate or glyphosate herbicide was used because both produced an initial defoliation. I compared their survival with survival of 13 cohorts that had not experienced herbicide treatment, choosing treatment and non-treatment cohorts to control for the type of crop field and weekly interval during the season. The herbicide-treatment cohorts were from 6 cornfields and 7 soybean fields, and the



**Figure 14.3.** Dates when herbicide is likely to be sprayed relative to monarch phenology. B = soybeans, C = corn, ng = nonglyphosate herbicide, g = glyphosate herbicide. Monarch phenologies for 4 seasons are shown, each based on average eggs per stem over all study sites per year.

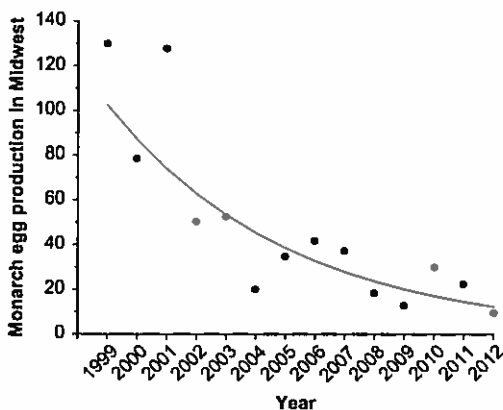
non-treatment cohorts were from 8 cornfields and 5 soybean fields. For each sampling interval from 23 May to 10 July, there were as many treatment cohorts as non-treatment cohorts. For the 157 eggs in the herbicide-treatment cohorts, 3 L2 larvae were found in the next census (1.9%). For the 144 eggs in the non-treatment cohorts, 25 L2 larvae were found in the next census (17.4%). The difference is statistically significant ( $P < 0.001$ , one-tailed Fisher's exact test based on the prediction that survival would be lower with herbicide application).

Pleasants and Oberhauser (2012) examined the effect of glyphosate herbicide use on milkweed and monarch populations in the U.S. Upper Midwest from 1999 to 2010. We used two surveys of milkweed densities in Iowa, one from 1999 (Hartzler and Buhler 2000) and the other from 2009 (Hartzler 2010) to gauge how milkweed densities had changed. These densities were converted to the area occupied by milkweeds in those habitats across Iowa using USDA land-use information. I have extended that analysis through 2012 and corrected a mistake in Pleasants and Oberhauser (2012). In that paper we used for our analyses the milkweed densities for

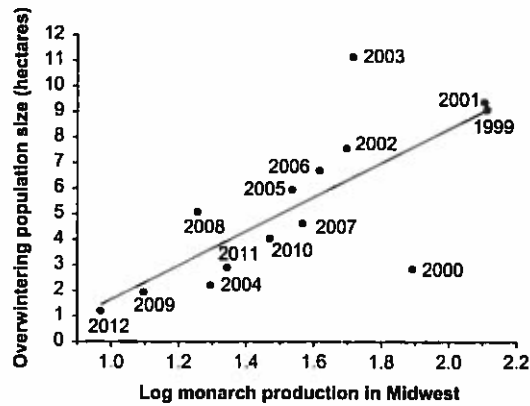
different habitats given in the tables of Hartzler and Buhler (2000) and Hartzler (2010); however, those densities were only for sample plots that had milkweeds. To calculate the overall density of milkweeds, we should also have included the zeroes from plots that had no milkweeds. The table in Hartzler (2010) shows that the proportion of crop fields infested with milkweeds went from 51% in 1999 to 8% in 2009 and that milkweed density in infested fields dropped from 23 (average in corn and soybeans) to 5  $\text{m}^2/\text{ha}$ . Thus the overall crop-field milkweed density should be 11.73  $\text{m}^2/\text{ha}$  for 1999 and 0.4  $\text{m}^2/\text{ha}$  for 2009, a decline of 96.5%. I have now redone tables 1 and 3 from Pleasants and Oberhauser (2012) with the corrected milkweed density values for different habitats. Based on this analysis I estimate that between 1999 and 2012, 98.7% of milkweeds in agricultural fields were eliminated, and that there has been a 64% reduction in milkweeds on the Iowa landscape, 72% of the loss occurring in agricultural fields. This loss is probably representative of the loss of milkweeds throughout the agriculturally intense landscapes of the Midwest as a whole (Pleasants and Oberhauser 2012); however, because monarch egg densities are

about 4 times higher per milkweed stem in agricultural fields relative to nonagricultural habitats (Pleasants and Oberhauser 2012), the loss of agricultural milkweeds represents an even greater loss of potential resource than the 64% reduction in milkweeds would indicate.

Yearly monarch production in the Midwest can be estimated by combining an indicator of the number of milkweed stems on the landscape each year (density in  $m^2/ha$ ) with the number of monarch eggs per milkweed stem, obtained from the Monarch Larva Monitoring Project (MLMP 2013; see Pleasants and Oberhauser 2012 for assumptions used in this estimate). Figure 14.4 shows Midwest monarch production estimates for 1999 through 2012. Based on the regression equation it is estimated that an 88% reduction in Midwest monarch production has occurred over this period. A decline is also seen in the size of the overwintering population (Brower et al. 2012a). Based on the overwintering numbers from 1999 to 2012 (Rendón-Salinas and Tavera-Alonso 2013), I performed a regression that indicated a population size decline of 72%. The similarity in the percent decline in Midwest monarch production and the percent decline in the size of the overwintering population suggests the two are related. In addition, Pleasants and Oberhauser (2012) found a strong correlation between Midwest production of monarchs in each year and the size of



**Figure 14.4.** Estimates of monarch numbers produced in the Midwest since 1999. The units for egg production are relative; they are the product of milkweed density in amount of cover ( $m^2$  per hectare) and eggs per milkweed plant (see Pleasants and Oberhauser 2012 for details). The regression line is based on the log of production:  $F_{1,13} = 34.35$ ,  $P = 0.00008$ ,  $r^2 = 0.74$ ,  $y = 2.08 - 0.071x$  where  $x = 1$  for 1999.



**Figure 14.5.** Monarch production in the Midwest vs. size of the overwintering population. Regression statistics:  $F_{1,13} = 14.89$ ,  $P = 0.002$ ,  $r^2 = 0.55$ ,  $y = -5.02 + 6.67x$ .

the overwintering population, suggesting a causal relationship between loss of milkweeds, declining midwestern monarch production, and a decline in the overall monarch population. Extending that analysis through 2012 further strengthens the correlation (Figure 14.5). While other factors such as loss of wintering habitat and persistent drought in Texas probably have contributed to the monarch population decline, the loss of milkweeds in the Midwest appears to be the primary cause.

## MILKWEED ABUNDANCE PRIOR TO AGRICULTURE

Extensive agricultural fields in the Midwest were not present before European settlement, so it is of interest to speculate on the amount of milkweed that might have been available to monarchs in pre-settlement times and whether the size of the monarch population that could be supported then was larger or smaller than today. No historical records exist that would provide such information, so we must rely on indirect estimates. Prairie remnants contain a vestige of pre-settlement vegetation, and the abundance of milkweed on these sites could be used to estimate pre-settlement abundance. White (1983) surveyed a number of prairie remnants in Iowa and quantified the percent cover of all plant species present according to plant community type. I used soil types as a proxy for community types (White 1983) and estimated the area occupied by soil types in Iowa (Oschwald et al. 1965). I then used

information on the percent cover of milkweed species in each community type to estimate milkweed cover values for the area of the state that was formerly prairie and is now in farmland.

White (1983) found three milkweed species in the remnant prairies: *A. viridiflora*, *A. verticillata*, and *A. tuberosa* (note the absence of *A. syriaca*). I estimated the overall percent cover of these three species as follows: *A. viridiflora* 0.11%; *A. verticillata* 0.22%; and *A. tuberosa* 0.32% (combined cover 0.65%). These data can be compared with estimates of the percent cover of the most common milkweed on the landscape, *A. syriaca*, using data from Pleasants and Oberhauser (2012), Hartzler and Buhler (2000), and Hartzler (2010). Milkweed is found in agricultural fields, CRP (Conservation Reserve Program) land, pastures, and roadsides. The percent cover of *A. syriaca* has decreased in all these habitats, except along roadsides, since 1999. In agricultural fields this reduction has been caused by glyphosate use; in the other habitats the reasons are less clear (Pleasants and Oberhauser 2012). In agricultural fields, percent cover of *A. syriaca* was 0.11% in 1999 but had declined to 0.0015% by 2012. The percent cover on CRP land has gone from 1.42% to 0.71% and on pastures, 0.04% to 0.02%. For roadsides, the percent cover has remained fairly constant at 0.76% (Hartzler 2010). Combining percent cover information with the amounts of land in each of these categories on the Iowa landscape, I estimate that the overall percent cover of *A. syriaca* went from 0.194% in 1999 to 0.068% in 2012.

It appears that the milkweed percent cover in 1999 was about a third of that based on historical prairie remnants, and current percent cover is about 10% of historical estimates. Interpreting these data with regard to the availability of milkweed resources for monarchs and the size of the monarch population during "pre-European settlement" versus "today" requires some caution. The data from prairie remnants may not be representative of the presettlement prairie vegetation in Iowa. The nine remnants surveyed by White (1983) include the largest ones in the state (68–97 ha), but the locations do not provide a representative coverage of the state. Another complicating factor is that in terms of their resource potential for monarchs, it is difficult to compare *A. viridiflora*, *A. verticillata*, and *A. tuberosa*, the three milkweed species found in the vegetation surveys of remnants, with *A. syri-*

*aca*, the predominant species used by monarchs today (Seiber et al. 1986). Monarchs lay eggs on *A. viridiflora*, *A. verticillata*, and *A. tuberosa* and larvae can complete their lives on these species (Orley Taylor, pers. comm.), but they might differ from *A. syriaca* in their attractiveness to ovipositing females and larval survival (Ladner and Altizer 2005) owing to differences in cardenolide and latex content (Zalucki et al. 1990; Zalucki and Malcolm 1999; Sternberg et al. 2012; Agrawal et al., this volume, Chapter 4). We also know that *A. syriaca* plants in agricultural fields have higher egg densities than *A. syriaca* plants in nonagricultural areas.

*A. syriaca* undoubtedly increased in abundance after European settlement as a result of the disturbance associated with agriculture. The primary pre-settlement habitat for midwestern *A. syriaca* was probably excavated soil around badger mounds or other animal burrows; it is rare in undisturbed prairie (Platt 1975) but is well adapted for colonizing disturbed areas (Wilbur 1976). In the eastern United States, milkweed abundance is certainly higher today than it was pre-European settlement as a result of clearing of forests for agriculture and habitation, and creation of disturbance habitat (Brower 1995); thus, milkweeds have likely been added in the eastern United States, while their numbers in the Midwest have remained similar or more recently decreased. How abundant were monarchs in pre- or early settlement times? Brower (1995) points out anecdotal observations from the 1850s and 1860s indicating large numbers of fall migrating monarchs, but there is no way to quantify these observations to compare them with current monarch numbers.

#### EXPLANATIONS FOR HIGHER EGG DENSITY ON AGRICULTURAL MILKWEEDS

One reason for the importance of agricultural milkweeds to monarchs is the higher density of eggs per milkweed stem in agricultural fields compared with stems in nonagricultural habitats. Several potential explanations can be given for the difference in egg density that are not mutually exclusive: egg survival might be higher in agricultural habitats because of less predation or more favorable microclimate; ovipositing monarchs might be more attracted from a distance to milkweeds in agricultural fields; or females might be more likely to lay

eggs on plants in agricultural fields once they find them. With regard to egg survival, Oberhauser et al. (2001) found that early-instar survival was higher in corn than in nonagricultural habitats (survival in soybeans was not systematically examined). With regard to overall attraction, Zalucki and Suzuki (1987) found that monarch females preferred ovipositing in small patches and on isolated plants, similar to what is found in agricultural fields (J. Pleasants, pers. observ.). In addition, the ability to find milkweed plants in monoculture crop fields could be greater than in mixed vegetation habitats because of the lower structural and chemical diversity of the vegetation (Floater and Zalucki 2000; Jactel et al. 2011).

With regard to the greater likelihood of oviposition on agricultural milkweeds once the plants are found, females might be responding to higher leaf quality. Milkweed plants in cornfields are shaded by the corn canopy, as are the lower halves of plants in soybean fields. Agrawal et al. (2012a) found that in shaded habitats *A. syriaca* leaves were larger, less tough, and had lower cardenolide content and lower induced latex production, possibly increasing their quality for monarch larvae (Oyeye and Zalucki 1990). Also, egg densities on milkweeds with young or resprouted leaves tend to be higher than on old leaves (Zalucki and Kitching 1982a), so resprouting after nonglyphosate herbicide application may also be partly responsible for the higher egg densities on milkweeds in agricultural fields. Some butterflies oviposit more on leaves with higher nitrogen (Letourneau and Fox 1989; Prudic et al. 2005), although a study involving monarchs found that leaf nitrogen concentration did not influence oviposition (Oyeye and Zalucki 1990), and plant nitrogen levels do not appear to affect monarch growth, since larvae are able to compensate for lower plant nitrogen by eating more (Lavoie and Oberhauser 2004). In a preliminary study in late July 2002, I analyzed nitrogen levels of leaves of milkweed plants growing in one cornfield, one soybean field, and several nonagricultural areas (two roadsides and one pasture) (9–12 leaves per site) using a CE Flash 1112 auto-analyzer (CE Elantech, Inc.). There were significant differences in percent nitrogen among the corn and soybean fields and the nonagricultural sites ( $F = 29.29$ ,  $df = 2,51$ ,  $P < 0.001$ ). There was a significantly higher percent nitrogen in leaves of milkweeds from soybean fields (mean = 4.8%) than cornfields (mean

= 3.9%) and both were higher than nonagricultural areas (mean = 2.7%) ( $t$ -tests significant for all with  $P < 0.006$  or smaller; significance based on Bonferroni corrected alpha value criterion of 0.017). Although Oyeye and Zalucki (1990) did not find an oviposition preference with leaf nitrogen, their highest observed nitrogen level was below the mean levels for corn and soybeans.

## CONCLUSIONS

Agriculture has clearly played, and continues to play, a significant role in monarch population biology, largely through its effect on the abundance of milkweeds. Historically agriculture in the eastern United States increased the abundance of *A. syriaca* over its original abundance, and agriculture in the Midwest also increased the abundance of *A. syriaca*, compensating, or perhaps more than compensating, for the loss of other milkweed species in the native prairie that it replaced. Within the last decade, however, the use of glyphosate herbicide, made possible by the widespread adoption of genetically modified corn and soybeans, has drastically reduced milkweed abundance in the Midwest. Milkweed abundance sets a ceiling on the size of the monarch population that can be supported, and that ceiling has been lowered substantially. Year-to-year variation in monarch population size is influenced by many factors, most notably weather conditions, but that variation plays out under declining food resources, as seen in Figure 14.4. The overall result is a decline in the size of the monarch population (Brower et al. 2012a; Pleasants and Oberhauser 2012), and this lower ceiling reduces the extent to which the monarch population can rebound from adverse events.

With the disappearance of milkweeds from agricultural fields, milkweeds present in other habitats become more important for monarch populations. The habitat of greatest importance is CRP land, potential agricultural land that has been set aside and planted with a cover of grasses and forbs. In Iowa, 59% of available milkweeds are now found in CRP land. This land is part of the agricultural system. The amount of CRP land is determined by government policies and the relative economic value of taking the government subsidy versus growing crops. In 2012 the amount of CRP land for the Midwestern states (North and South Dakota,



Nebraska, Kansas, Minnesota, Iowa, Missouri, Wisconsin, Illinois, Michigan, Indiana, Ohio) had declined by 1.56 million hectares from its high in 2007 of 7.12 million hectares (USDA, Conservation Programs 2013). With the lowering of the ceiling brought about by the disappearance of milkweed in crop fields, it is important that further lowering not occur through the loss of CRP land. It is possible, however, to enhance the value of CRP land for monarchs as well as other species such as pollinators through appropriate management practices (e.g., Baum and Mueller, this volume, Chapter 17). The next most important habitat is roadsides, accounting for 36% of milkweeds in Iowa. Department of

Transportation practices, such as the timing of mowing and herbicide spraying, need to be examined for their effect on monarch production.

#### ACKNOWLEDGMENTS

I thank Karen Oberhauser, Chip Taylor, and Lincoln Brower for discussions and data sharing. The data on monarch egg densities used in the analyses of the relationship between overwintering numbers and monarch production in the U.S. Upper Midwest were collected by volunteers in the Monarch Larva Monitoring Project.



# NATIONAL STRATEGY TO PROMOTE THE HEALTH OF HONEY BEES AND OTHER POLLINATORS

Pollinator Health Task Force

MAY 19, 2015





May 19, 2015

On behalf of the Pollinator Health Task Force, we are pleased to transmit the *National Strategy to Promote the Health of Honey Bees and Other Pollinators* (Strategy). Developed through a collaborative effort across the Executive Branch, this Strategy outlines a comprehensive approach to tackling and reducing the impact of multiple stressors on pollinator health, including pests and pathogens, reduced habitat, lack of nutritional resources, and exposure to pesticides. Building on the current state of the science, and with a renewed emphasis on expanding our understanding of the complex interactions among the various factors impacting pollinator health, the Strategy lays out current and planned Federal actions to achieve the following overarching goals:

- **Honey Bees:** Reduce honey bee colony losses during winter (overwintering mortality) to no more than 15% within 10 years. This goal is informed by the previously released Bee Informed Partnership surveys and the newly established quarterly and annual surveys by the USDA National Agricultural Statistics Service. Based on the robust data anticipated from the national, statistically-based NASS surveys of beekeepers, the Task Force will develop baseline data and additional goal metrics for winter, summer, and total annual colony loss.
- **Monarch Butterflies:** Increase the Eastern population of the monarch butterfly to 225 million butterflies occupying an area of approximately 15 acres (6 hectares) in the overwintering grounds in Mexico, through domestic/international actions and public-private partnerships, by 2020.
- **Pollinator Habitat Acreage:** Restore or enhance 7 million acres of land for pollinators over the next 5 years through Federal actions and public/private partnerships.

The Strategy addresses the four themes central to the June 2014 Presidential Memorandum “Creating a Federal Strategy to Promote the Health of Honey Bees and Other Pollinators,” namely: conducting research to understand, prevent, and recover from pollinator losses; expanding public education programs and outreach; increasing and improving pollinator habitat; and developing public-private partnerships across all these activities. A critical component of the Strategy is to advance the science underpinning the government’s land management and regulatory decisions. To this end, the Task Force has prepared the accompanying “Pollinator Research Action Plan,” which outlines gaps in current knowledge of pollinators and pollinator declines, and identifies priority research efforts needed to close these gaps.

The Strategy also advances ambitious Federal commitments to increase and improve habitat for pollinators, both directly through the large variety of facilities and acreages of land managed by the Federal government, and indirectly through the leadership role that Federal agencies can play in interactions with states, localities, the private sector, and citizens. These actions range from planting pollinator gardens and improving land management practices at Federal facilities, to advancing the availability and use of pollinator-friendly seed mixes in land management, restoration, and rehabilitation actions nationwide.

By expanding the conversation through enhanced public education and outreach, as well as strongly-built public/private partnerships, the Strategy seeks to engage all segments of our society so that, working together, we can take meaningful and important steps to reverse pollinator declines.

Pollinators are critical to our Nation's economy, food security, and environmental health. Honey bee pollination alone adds more than \$15 billion in value to agricultural crops each year, and provides the backbone to ensuring our diets are plentiful with fruits, nuts, and vegetables. Through the actions discussed in this Strategy, and by working with partners across our country, we can and will help restore and sustain pollinator health nationwide.

A handwritten signature in black ink, appearing to read "Tom Vilsack".

Hon. Tom Vilsack  
Secretary of Agriculture

A handwritten signature in black ink, appearing to read "Gina McCarthy".

Hon. Gina McCarthy  
Administrator, U.S. Environmental Protection Agency





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## Executive Summary

Wherever flowering plants flourish, pollinating bees, birds, butterflies, bats, and other animals are hard at work, providing vital but often unnoticed services. But many pollinators are in serious decline in the United States and worldwide. Preventing continued losses of our country's pollinators requires immediate national attention, as pollinators play a critical role in maintaining diverse ecosystems and in supporting agricultural production. Some three-fourths of all native plants in the world require pollination by an animal, most often an insect, and most often a native bee. Pollinators, most often honey bees, are also responsible for one in every three bites of food we take, and increase our nation's crop values each year by more than 15 billion dollars. Unabated, these losses of our pollinators threaten agricultural production, the maintenance of natural plant communities, and the important services provided by those ecosystems, such as carbon cycling, flood and erosion control, and recreation.

In response to this threat, in June 2014, President Obama issued a memorandum establishing a Pollinator Health Task Force, co-chaired by the Secretary of Agriculture and the Administrator of the Environmental Protection Agency. The Task Force created this document, the *National Strategy to Promote the Health of Honey Bees and Other Pollinators* (Strategy), to promote the health of honey bees (*Apis mellifera*) and other managed bees, wild bees (both native and introduced species), butterflies and other pollinating insects, and birds and bats.

The Strategy expands and adds to actions already being undertaken by Federal departments and agencies to reverse pollinator losses and restore populations to healthy levels. It focuses on both immediate and long-term changes that can be made to improve the well-being of pollinator populations. Consequently, the Strategy addresses the many factors impacting pollinator health, including certain land-use practices, declining forage and nesting resources, pests and diseases, pesticides, and bee biology.

While our nation is a mosaic of land uses and ownerships, pollinating animals do not recognize human-drawn boundaries. They make use of food and habitat anywhere it is found, whether on national park land, a roadside strip, the edge of an agricultural field, or a schoolyard garden. Therefore, no single organization, Federal or private, can independently shoulder the burden of helping pollinators, and the Task Force has been charged with an "all hands on deck" approach to promoting the health of honey bees and other pollinators.

The Strategy knits together commitments and plans from many Federal departments and agencies, bringing a variety of missions and programs to bear toward a single, unified goal—promoting the health of the nation's pollinators. The Federal government is the largest land manager in the Nation and through its programs can also coordinate with private sector actions. In response to the Presidential Memorandum, land management agencies are identifying lands to manage for new and better pollinator habitats: the U.S. Environmental Protection Agency (EPA) is working to balance the unintended consequences of chemical exposure with the need for pest control; the U.S. Department of Agriculture (USDA) is looking to expand pollinator habitats, particularly summer foraging areas, under the Conservation Reserve Program; and habitat opportunities are being found in new and creative places, such as on rights-of-way and other easements.

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One innovative approach with great potential is the inclusion of pollinator-friendly landscaping at Federal facilities. Beneficial landscaping and gardens are already in place at a number of Federal facilities, such as the Smithsonian Institution, the National Zoo, USDA, and the White House Pollinator Garden, with others being planned by the Departments of Transportation, Interior, Defense, and State, the EPA, and others.

This Strategy outlines three overarching goals for action by Federal departments and agencies in collaboration with public and private partners:

1. Reduce honey bee colony losses during winter (overwintering mortality) to no more than 15% within 10 years. This goal is informed by the previously released Bee Informed Partnership surveys and the newly established quarterly and annual surveys by the USDA National Agricultural Statistics Service. Based on the robust data anticipated from the national, statistically-based NASS surveys of beekeepers, the Task Force will develop baseline data and additional goal metrics for winter, summer, and total annual colony loss.
2. Increase the Eastern population of the monarch butterfly to 225 million butterflies occupying an area of approximately 15 acres (6 hectares) in the overwintering grounds in Mexico, through domestic/international actions and public-private partnerships, by 2020.
3. Restore or enhance 7 million acres of land for pollinators over the next 5 years through Federal actions and public-private partnerships.

To achieve these goals, the Task Force developed a series of action plans and resources. Underpinning these goals is the Pollinator Research Action Plan (PRAP 2015), designed to focus Federal efforts on producing the scientific information needed to understand, minimize, and recover from pollinator losses. Task Force agencies also developed pollinator Best Management Practice (BMP) guidance for Federal buildings and designed and natural landscapes. Federal agencies are identifying pollinator-beneficial plants that meet nutritional needs of honey bees and other pollinators. The National Seed Strategy for Rehabilitation and Restoration will develop a seed bank of appropriate plants to support restoration activities and to help ensure a stable, economical supply of diverse native plants.

Increasing the national awareness of the importance of pollinator conservation is addressed in agency plans for public outreach and education. These plans constitute a multifaceted portfolio of public education and outreach strategies for multiple audiences: individuals; small businesses and corporations; schools, libraries, museums, and other educational venues; demographically diverse audiences; and Federal land-management staff.

Understanding that the Federal government cannot act alone in promoting pollinator protection, the President also identified the need for public-private partnerships. The Strategy includes recommendations and guidance for developing public-private partnerships to build on Federal efforts encouraging the protection of pollinators and increasing the quality and quantity of pollinator habitat. The Task Force welcomes partnership ideas, and will prepare a Partnership Action Plan within six months of release of this Strategy.

As pollinator science matures and our information about pollinators becomes more robust, so too will the long-term Federal strategy. Specific goals and milestones are identified in the Strategy, along with associated timelines and metrics for evaluating the Strategy's success. Progress toward these goals and actions will be assessed and publicly disseminated annually.



## Introduction

Pollinators are crucial members of various ecosystems, from farmland to wilderness to urban environments. There are an estimated several hundred thousand flowering plant species, many of which depend on pollinators to reproduce (National Research Council 2007). A variety of animals serve as pollinators, *e.g.*, bees, wasps, flies, butterflies, moths, bats, beetles, and birds. The attributed value of crops that are directly dependent on insect pollination was estimated at \$15 billion in 2009 in the United States (Calderone 2012).

### Domestic Losses of Honey Bees

Honey bees, the most recognizable pollinators of hundreds of economically and ecologically important crops and plants in North America, are an introduced insect, brought to the United States in the 1620's by early settlers. Approximately 2,000-3,000 commercial<sup>1</sup> U.S. beekeepers manage their bee colonies as livestock, traveling across the country with their bees to service pollination contracts with U.S. farmers and to support honey production (Calderone 2012).

Honey bees have been in serious decline for more than three decades in the United States, as noted in the National Academy of Sciences report *Status of Pollinators in North America* (National Research Council, 2007). Declines in the number of managed honey bee colonies used in honey production have been documented by the USDA's National Agricultural Statistics Service (USDA 2014). Starting in the 1940's when there were approximately 5.7 million colonies in the United States, the number of managed colonies used in honey production has declined to approximately 2.74 million colonies today (**Figure 1**). Sharp colony declines were seen following the introduction in 1987 of an external parasitic mite (*Varroa destructor*) that feeds on honey bee hemolymph (blood), and again around 2006 with the first reports of a condition referred to as Colony Collapse Disorder (CCD). Colonies diagnosed with CCD exhibit a rapid loss of adult worker bees, have few or no dead bees present in the colony, have excess brood and a small cluster of bees remaining with the queen bee, and have low *Varroa* mite and *Nosema* (fungal disease) levels. Colonies exhibiting CCD have insufficient numbers of bees to maintain the colony (*e.g.*, rearing and maintenance of developing young, food collection, and hygiene) and these colonies eventually die. Although CCD has become synonymous with all honey bee colony declines, the actual proportion of losses directly attributable to CCD is low and has been decreasing over the past four years, based on beekeeper winter loss surveys conducted by the Bee Informed Partnership, supported by the USDA (Steinhauer *et al.* 2014).

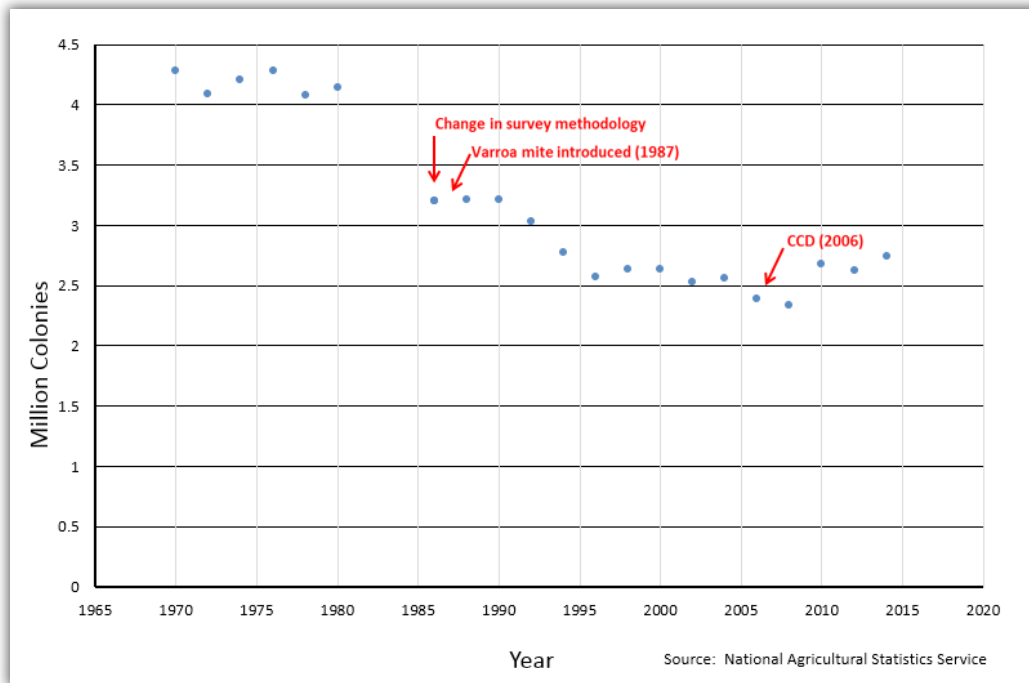
Although **Figure 1** indicates that the number of managed honey bee colonies has been relatively consistent since 1996, the level of effort by the beekeeping industry to maintain these numbers has increased. Annual surveys of beekeepers since 2006 indicate overwintering losses alone averaging around 31% (**Figure 2**), which far exceeds the 15-17% overwintering loss rate that commercial beekeepers have indicated is an economically sustainable average (Steinhauer *et al.* 2014). When overwintering losses are coupled with colony losses occurring during other times of the year, annual losses can be considerably higher (Steinhauer *et al.* 2014). This is particularly notable in the 2014-15 preliminary report of 27.4%

1. The American Beekeeping Federation classifies beekeepers based on the number of honey bee colonies they maintain: small scale (<25 colonies), sideline (25 – 300 colonies), and commercial (>300 colonies).

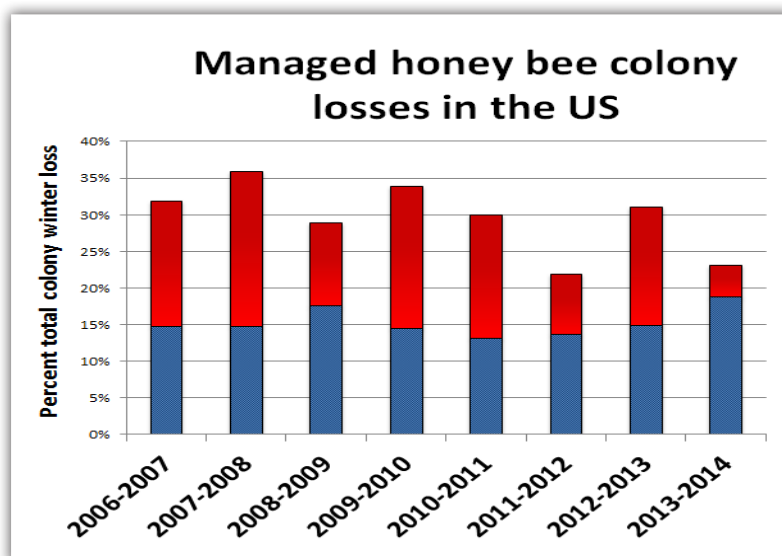


## NATIONAL STRATEGY TO PROMOTE THE HEALTH OF HONEY BEES AND OTHER POLLINATORS

total summer colony losses in the Bee Informed Partnership survey of a subset of national beekeepers, for total annual losses of 42.1% of colonies (Steinhauer et al. 2015).



**Figure 1.** Numbers (in millions) of managed honey bee colonies in the United States used for honey production by year based on NASS survey data. The gap between 1982–1986 reflects the period when the survey was not conducted. The figure illustrates when the Varroa mite was introduced into the United States in 1987, and when Colony Collapse Disorder was first documented in 2006.



**Figure 2.** Annual overwintering losses of managed honey bee colonies (October 1–April 1; red bars), and self-declared acceptable mortality level from participant beekeepers (blue bars). Bee Informed Partnership 2014 (<http://beeinformed.org/2014/05/colony-loss-2013-2014/>).

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Meeting the growing demand for pollination services in agricultural production has become increasingly difficult. Beekeepers transport bees long distances to pollinate crops such as apples, blueberries, cherries, squash, and, particularly, almonds. Approximately 60–75% of all U.S. commercial honey bee colonies are required in almond orchards early each spring to fulfill pollination contracts (Bond *et al.* 2014). When overwintering colony losses are high, beekeepers must compensate for these losses by “splitting” one colony into two, supplying the second colony with a new queen bee and supplemental food in order to quickly build up colony strength to fulfill almond pollination contracts. This practice results in increased maintenance costs to both the beekeeper and the orchard grower renting the hives, with hive rental fees for almond pollination rising from approximately \$76 per hive in 2005 to over \$150 per hive in 2009 (Bond *et al.* 2014).

Researchers studying CCD and other losses attributed to poor colony health have been unable to identify a single cause, and have concluded that losses of honey bee colonies are the result of a complex set of interacting stressors. In May 2013, the USDA and the EPA released a comprehensive scientific report on honey bee health (USDA 2013). The report synthesized the current state of knowledge regarding the primary factors that scientists believe have the greatest impact on honey bee health, including exposure to pesticides and other environmental toxins, poor nutrition due in part to decreased availability of high-quality/diverse forage, exposure to pests (*e.g.*, Varroa mites) and disease (viral, bacterial, and fungal), as well as bee biology, genetics, and breeding. The report’s findings are similar to those of the report on the *Status of Pollinators in North America* (NRC 2007), which examined wild (both native and introduced species) pollinators as well as honey bees.

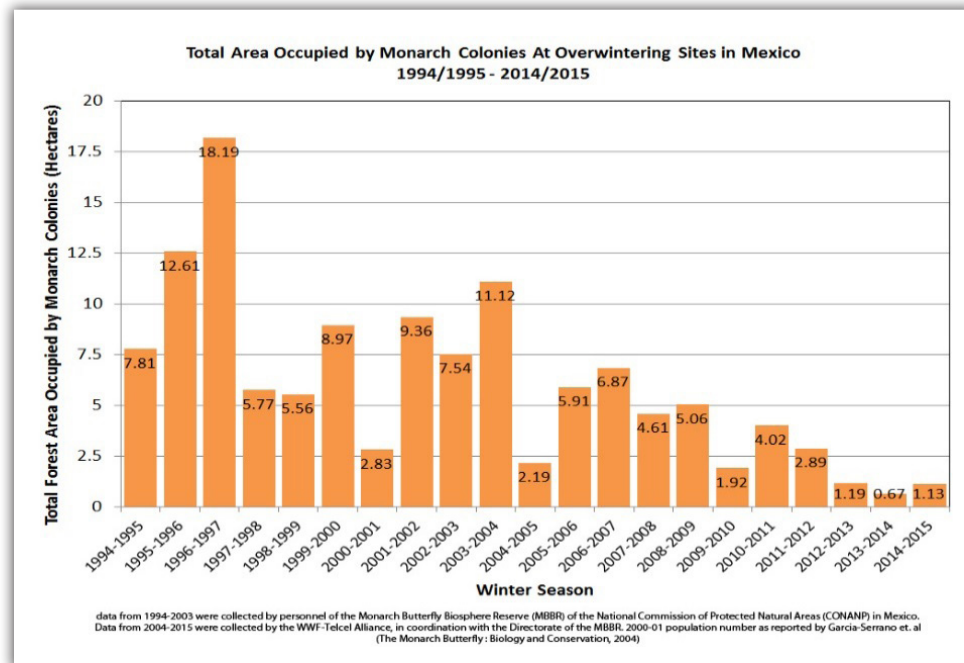
### Domestic Losses of Other Pollinators

In addition to honey bees, there are over 4,000 wild bee species in the United States (Moisset and Buchmann 2011). Population declines in the United States have been documented for some populations of non-managed pollinators, *e.g.*, the two-formed bumble bee (*Bombus bifarius*) (Spivak *et al.* 2011; Cameron *et al.* 2011), but little is known about trends for populations of non-managed bees that comprise the majority of pollinators (Winfrey *et al.* 2007; Lebuhr *et al.* 2013). Some bumble bee populations are suffering from introduced pests and diseases, potentially transferred from managed bees (Colla *et al.* 2006; McMahon *et al.* 2015). Non-*Apis* bees, butterflies, bats, and other managed or wild pollinators are also impacted by habitat loss and degradation, and there is strong evidence that, for some species, habitat loss has led to population declines (NRC 2007; Potts *et al.* 2010). All pollinators must also cope with the effects of climate change, which may have direct impacts on behavior and physiology, and indirect impacts through floral resource availability and phenology, as well as changing dynamics of pests, pathogens, predators, and competitors (Potts *et al.* 2010; Le Conte and Navajas 2008).

As with honey bees and other managed or wild bee pollinators, there have been marked (~90%) declines in monarch butterfly (*Danaus plexippus*) populations over the past several years (**Figure 3**). In February 2014, President Obama committed to work together with Canadian Prime Minister Stephen Harper and Mexican President Enrique Peña Nieto to ensure the conservation of the monarch butterfly. Much of a monarch butterfly’s life is spent completing part of an annual cycle of migration over the course of multiple generations, either across North America between Canada into Mexico (Eastern migration), or between the Rocky Mountains and groves in California (Western migration). The iconic Eastern migra-

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tion, in particular, has become less successful for many monarchs because of losses in nectar-producing plants that provide sustenance to the adult butterflies, as well as in the availability of milkweed plants on which developing monarch larvae feed exclusively. Primary stressors of concern for the Eastern population include loss of milkweed breeding habitat in corn and soybean production, loss of breeding habitat due to land conversion, illegal logging and deforestation at overwintering sites, and extreme weather conditions. Natural enemies such as diseases, predators, and parasites, and use of insecticides in agricultural, urban, and suburban areas are also of concern.



**Figure 3:** Area of forest occupied by colonies of hibernating monarch butterflies in Mexico from 1994–2015 (Graph courtesy of the Monarch Joint Venture).

Determining the current status of insect pollinator communities, documenting shifts in distribution and abundance of various species, and refining methodologies for documenting changes remain important areas of research (Lebuhn *et al.* 2013), along with developing taxonomic capacity to identify the thousands of North American bee species. Additional research is also needed on the value of pollinators in natural systems, which is much more difficult to discern than for managed honey bees. The economic value of managed non-*Apis* bees, *e.g.*, blue orchard bees (*Osmia lignaria*), alfalfa leafcutting bees (*Megachile rotundata*), bumble bees (*Bombus spp.*), etc., has not been well-quantified, despite the fact that these species are highly effective crop pollinators. Wild, native bees also provide the majority of pollination that helps maintain natural plant communities which contribute to a variety of valuable ecosystem services, including carbon sequestration, water filtration, and erosion control (NRC 2007). Simultaneous declines in wild and managed pollinator populations globally, with noted decreases in honey bees, bumble bees, and monarch butterflies, have brought into focus the importance of pollinator conservation (Cameron *et al.* 2011; NRC 2007; Pettis and Delaplane 2010; vanEngelsdorp *et al.* 2009).

## International Considerations

Declines in honey bees, wild bees, and other pollinators are not unique to the United States. Across the globe, similar patterns of decline in wild and managed pollinator populations have been documented over similar timespans (Biesmeijer *et al.* 2006). From 1985–2005, the number of managed honey bee colonies declined in many countries in Europe, along with marked declines in beekeepers (Potts *et al.* 2010). A number of international organizations have undertaken efforts to better understand the causes and magnitude of pollinator population declines. Such global activities, including the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES), as well as efforts through the International Bee Research Association (IBRA), inform and are informed by work being undertaken in the United States. Federal agencies, such as the USDA and the EPA, are working with their counterparts in the Organisation for Economic Cooperation and Development (OECD) and with researchers internationally through the International Commission on Plant-Pollinator Relationships (ICPPR) and the Colony Loss (COLOSS) Network to understand the factors associated with global declines in pollinator species and how these declines can be mitigated. International cooperation, financially and scientifically, leverages U.S. investments with investments being made by other countries, and also provides an opportunity for the United States, with its diversity of ecosystems and large Federal and Federally-funded research community, to contribute to solving this global challenge.

In many countries, estimates for pollinator populations and the magnitude of different possible stressors are not available for comparison to what is being experienced in this country. The forthcoming IPBES assessment on pollination, pollinators, and food production, due to be completed in 2015, may reveal other sources of information or significant international gaps in understanding the magnitude of losses and the potential consequences if left unchecked. This assessment will also address monetary and non-monetary ecosystem services provided by pollinators across the globe.



## Establishment of the Pollinator Health Task Force

Given the breadth, severity, and persistence of pollinator losses, President Obama issued his June 20, 2014 Presidential Memorandum, “Creating a Federal Strategy To Promote the Health of Honey Bees and Other Pollinators” (FR Doc. 2014-14946; White House 2014), to the heads of Federal departments and agencies, calling for the creation of a Federal strategy to promote the health of honey bees and other pollinators. Citing the critical roles that pollinators play in contributing to the economy, providing a nutritious supply of fruits, nuts, and vegetables, and maintaining a variety of valuable ecosystem services, the President charged Federal departments and agencies with taking steps to reverse pollinator losses and to help restore pollinator populations. The Federal government is poised to lead this effort, given its broad national perspective and ability to identify and prioritize goals and programs that extend beyond state and national borders. Understanding that the Federal government cannot act alone in promoting pollinator protection, the President also identified the need for public-private partnerships as well as increased citizen engagement.

To accomplish this effort, the President created the Pollinator Health Task Force, co-chaired by the Secretary of Agriculture and the Administrator of the Environmental Protection Agency. In addition to USDA and EPA, the Task Force was chartered to include representation from the following departments and agencies:

- Council on Environmental Quality (CEQ);
- Department of Defense (DOD);
- Department of Education (ED);
- Department of Energy (DOE);
- Department of Housing and Urban Development (HUD);
- Department of the Interior (DOI);
- Department of State (DOS);
- Department of Transportation (USDOT);
- Domestic Policy Council (DPC);
- General Services Administration (GSA);
- National Science Foundation (NSF);
- National Security Council (NSC);
- Office of Management and Budget (OMB);
- Office of Science and Technology Policy (OSTP); and,
- Such executive departments, agencies, and offices as the Co-Chairs may designate.



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Since its initial formation, the Task Force has expanded to include representatives from the Smithsonian Institution (SI) and the Federal Emergency Management Agency (FEMA).

To advance the state of knowledge used to inform pollinator protection efforts through interagency collaboration, the Task Force developed a Pollinator Research Action Plan (PRAP 2015) and Pollinator-Friendly Best Management Practices for Federal Lands (USDA/DOI 2015), to assist agencies in developing and enhancing pollinator habitat. The Task Force also oversaw the development of agency public education and outreach plans. The *National Strategy to Promote the Health of Honey Bees and Other Pollinators* (Strategy) is comprised of these materials, with an emphasis on public-private partnerships. The Strategy addresses the key stressors that impact pollinator health, notably: (1) nutrition, with a focus on providing adequate forage resources for pollinators; (2) land-use policies and practices to increase forage and nesting resources for a variety of pollinators; (3) management of arthropod pests and disease pathogens; (4) pesticides; and (5) rearing issues, including bee biology, genetics, and breeding. To be successful in reversing pollinator declines, it is vital that the Strategy address all of the above factors and the complex interactions between each of these factors that are likely contributing to declines.

The Presidential Memorandum empowers the Task Force to move forward with a broad range of activities and partnerships that collectively are intended to reverse pollinator declines. The Strategy focuses on both immediate changes that can be made to improve pollinator health, consistent with the best-available science to support these actions, as well as efforts to improve pollinator health over the long term. In implementing the Strategy, Federal agencies will lead by example and will also more fully engage public and private partners in academia, non-governmental organizations, private industry, state and local governments, foundations, and private citizens.



# Development of the National Pollinator Health Strategy

The Presidential Memorandum instructed the Task Force to develop a National Pollinator Health Strategy that incorporates research and development, outreach, and public-private partnerships. In addition, building on agency-specific actions, either identified in the Presidential Memorandum or through enhanced actions by individual agencies, the Strategy seeks to identify opportunities and initiatives for addressing both short-term and long-term habitat improvement that will benefit overall pollinator health. Through revised guidance, Federal contracting procedures, and regulatory actions, a priority outcome of this Strategy is to institutionalize changes into Federal initiatives to ensure that pollinator health actions have longevity and lead to continuing improvement. While the focus of the Strategy is on improving pollinator health, many of the recommendations identified in the Strategy will also have collateral benefits in improving ecosystems more broadly, through encouraging development and maintenance of native habitats and more ecologically sustainable land management practices. This is especially true for efforts to protect the monarch butterfly, which is a minor pollinator but a major indicator of biodiversity and ecosystem health.

## Target Outcomes

A key to the Strategy is the inclusion of metrics for measuring successes and to identify the need to adjust actions in advancing the Strategy's goal, which is to restore the health of affected pollinator species and prevent further unacceptable declines. Success will be assessed through three outcome metrics: (1) returning honey bee colony health to acceptable levels (approximately 15% overwintering loss, a level from which beekeepers are capable of successfully dividing surviving healthy colonies to remain economically viable); (2) increasing monarch butterfly populations to historic averages to ensure successful continuation of annual migrations; and (3) increasing and maintaining cumulative pollinator habitat acreage in critical regions of the country. Numeric outcome metrics are quantified in **Table 1**.

**Table 1. Overarching Pollinator Health Outcome Metrics**

<p><b>1. Honey Bees:</b> Reduce honey bee colony losses during winter (overwintering mortality) to no more than 15% within 10 years. This goal is informed by the previously released Bee Informed Partnership surveys and the newly established quarterly and annual surveys by the USDA National Agricultural Statistics Service. Based on the robust data anticipated from the national, statistically-based NASS surveys of beekeepers, the Task Force will develop baseline data and additional goal metrics for winter, summer, and total annual colony loss.<sup>a</sup></p>
<p><b>2. Monarch Butterflies:</b> Increase the Eastern population of the monarch butterfly to 225 million butterflies occupying an area of approximately 15 acres (6 hectares) in the overwintering grounds in Mexico, through domestic/international actions and public-private partnerships, by 2020.</p>
<p><b>3. Pollinator Habitat Acreage:</b> Restore or enhance 7 million acres of land for pollinators over the next 5 years through Federal actions and public/private partnerships.</p>

<sup>a</sup> Based on the success of research, it is hoped that overwintering losses would be further reduced to pre-Varroa mite levels.

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The outcome metrics identified in **Table 1** address the President's directive to expand Federal efforts to reverse pollinator losses and to help restore populations to healthy levels. Due to the critical importance of pollinators to the economy, including to agricultural production<sup>2</sup> and general ecosystem services, the ultimate objective of the Task Force is to ensure a level of pollinators that would sustain agricultural production and protect the health of the environment for the foreseeable future. In that context, the Task Force's first target outcome is to improve honey bee population health by reducing honey bee winter losses by no less than 50% from current levels, which have averaged around 30% nationwide over the past 5 years (Steinhauer *et al.* 2014), to a sustainable 15% loss rate within 10 years (by 2025). This reduction in the 5 year average of winter losses would be accomplished in stages: (1) by 25% (*i.e.*, to a 22% colony loss rate) by 2020; and, (2) by a total reduction of 50% by 2025. This overall reduction to 15% yearly winter losses would restore an economically sustainable system for beekeepers and growers who depend on pollination services. This is an ambitious goal and the Task Force recognizes that yearly fluctuations due to the impacts of unknowable and difficult-to-mitigate variables (*e.g.*, drought, severe winter weather, or new bee maladies) may result in losses in a given year that are higher than the target average. The Task Force also acknowledges, based upon ongoing research discussed in the PRAP (2015), the possibility of further reductions, perhaps to pre-Varroa mite levels.<sup>3</sup>

Summer losses also lead to cumulative economic stress on beekeepers, notably the 2014–15 preliminary colony loss results from the Bee Informed Partnership. In summer 2014 (April–October) the colony loss rate was reported at 27.4% among a subset of national beekeepers responding to the survey. Combined with overwintering losses, the total annual colony loss (April 1, 2014–March 30, 2015) was 42.1% (Steinhauer *et al.* 2015). Overwintering mortality data are based on a different survey respondent pool, and for 2014–15 overwintering mortality was reported at 23.1%. The summer and annual colony loss data were first included in the Bee Informed Partnership survey in 2010–11.

The Task Force's second target outcome is to increase the Eastern population of the monarch butterfly to 225 million butterflies occupying an area of approximately 15 acres (6 hectares) in the overwintering grounds in Mexico by 2020. This goal represents the approximate average winter population level from 1994–2014 and also assumes an estimated density of 37.5 million butterflies per hectare. The Eastern monarch population has experienced a significant decline over the past 20 years. The 2014–2015 overwintering count of 56.5 million butterflies for the Eastern population was the second-lowest count on record, representing a population decline of 82% from the 20-year average. The occupied overwintering habitat in 2014–2015 measured only 2.8 acres (~1.1 hectares).<sup>4</sup> The Task Force views a target of 225 million butterflies occupying an area of approximately 15 acres (6 hectares) for the Eastern migration

2. Honey bees alone are estimated to support the cultivation of 90 – 130 crops which directly or indirectly account for up to a third of the U.S. diet (Bond *et al.* 2014).

3. Winter colony loss has averaged 28% nationwide over the last five winters for which we have data (2009–2010 to 2013–2014. Estimates from the Bee Informed Partnership, [www.beeinformed.org](http://www.beeinformed.org)), compared to an estimated average annual loss of 15% prior to the arrival of the Varroa mite in 1987 and the sharp rise in Colony Collapse Disorder in 2006. Prior to 2006, there was no coordinated effort to collect data on winter survival nationwide. Estimates of 15% colony loss prior to 1987 are anecdotal from beekeepers and bee researchers. In 2006, the Bee Informed Partnership began collecting data on winter losses, as well as data on winter losses from beekeepers who felt their losses were "acceptable." Since 2006, the average self-reported rate of acceptable losses is 15%.

4. Data from Rendón-Salinas, E., A. Fajardo-Arroyo, and G. Tavera-Alonso. 2014. Forest surface occupied by monarch butterfly hibernation colonies in December 2014 World Wildlife Fund – Mexico report. Available from <https://www.worldwildlife.org/publications/forest-surface-area-occupied-by-monarch-butterfly-hibernation-colonies-in-december-2014>.

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as the best indicator of holistic species health. The Task Force is also mindful of the importance of the Western population in maintaining species viability across the continent.

The Task Force's third target outcome, restoration and enhancement of 7 million acres of pollinator-friendly habitat, addresses the importance of providing new and diverse nectar and pollen resources for honey bees and wild pollinators, including the monarch butterfly. Restoration of habitat is defined as the manipulation of the physical, chemical, or biological characteristics of a site with the goal of returning the majority of natural functions to the lost or degraded native habitat (16 USCS §3772 (5)); whereas habitat enhancement is defined as the manipulation of the physical, chemical, or biological characteristics of an undisturbed or degraded site to heighten, intensify, or improve specific functions or to achieve a specific purpose. As such, habitat enhancement represents a more targeted effort.

The habitat target outcome is based on preliminary expert estimates regarding the need to offset annual losses of pollinator habitat, plus provide additional acres to reverse past losses. These estimates are preliminary until comprehensive peer-reviewed literature becomes available to quantify the total magnitude of habitat losses, or needs for recovery. The estimates will be refined to reflect the findings of ongoing research in the PRAP (2015) to better measure pollinator status and acreage needs, and to identify those land areas and corridors most valuable and amenable to enhancement or restoration. For instance, the U.S. Geological Survey (USGS) Powell Center is working to identify habitats and corridors most valuable for directing resources for conservation of the Eastern population of the monarch butterfly, and the USDA is focusing Conservation Reserve Program (CRP) and Environmental Quality Improvement Program (EQIP) resources on the five upper Midwest States (South Dakota, North Dakota, Minnesota, Wisconsin, Michigan) that are central to honey bee summer forage. All actions will be subject to adaptive management as this research becomes available, in recognition of the fact that reversing pollinator losses is a long-term process requiring the incorporation of pollinator health considerations in routine agency and private-sector actions, rather than a one-off solution.

The habitat target outcome is also consistent with actions included by agencies in this Strategy. These actions include, but are not limited to: USDA resources applied to CRP and EQIP pollinator enhancements, and national forest and grassland acreage; DOI actions to restore or enhance lands through direct restoration action, along with the inclusion of pollinator-friendly native seeds in all post-fire revegetation and fuels/green stripping projects; U.S. Army Corps of Engineers (USACE) implementation of pollinator best management practices at its facilities; and numerous other actions itemized by Federal agencies to increase pollinator habitat. Federal agencies will also be working with the private sector to improve pollinator habitat on lands not managed by the Federal government, including state- and locally-managed lands, such as parks and highway rights-of-way, and privately-owned lands ranging from home gardens to corporate and philanthropically-sponsored acreage. The target outcome anticipates that fifty percent of acreage improvement will be sourced from Federally-managed lands, and fifty percent through working with partners to create or enhance habitat on state, locally-managed, and private lands.

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## Measuring Success

To achieve these target outcomes, each relevant action undertaken by a Federal agency will also include a timeline and metrics for evaluating the success and progress toward achieving one or more of these target outcomes. As the science developed through the Pollinator Research Action Plan (2015) matures, adjustments and/or enhancements to Federal actions and overarching goals and target outcomes also may be warranted. With expanding implementation of the Strategy, and as partnership efforts continue to grow, additional metrics and measures will be added to aid in assessing the success of the Strategy.

Periodic follow-up and reporting of agency performance is also vital in demonstrating to the public the Federal government's commitment to reversing pollinator declines and improving pollinator health. To this end, Task Force agencies are to report annually on all metrics to the Task Force Co-Chairs, who will publicly disseminate the results on an annual basis so that the general public can monitor the progress each agency is making in fulfilling the commitments detailed in this Strategy, including collaboration with public and private stakeholders.

## Budget Requests for Pollinator Health

The actions contemplated in this Strategy are not occurring *de novo* or in a vacuum. Considerable Federal resources are already being directed toward honey bee, monarch butterfly, and other pollinator health-related issues, and a number of significant documents have investigated these issues. For instance:

- In 2007, the National Research Council published its report emphasizing risks posed to pollinator populations, stimulating further action.
- A Federal action plan for honey bees, the 2007 Colony Collapse Disorder Action Plan (USDA 2007), built on existing knowledge and resource bases within agencies.
- The 2008 North American Monarch Conservation Plan was developed by a team of experts from Canada, Mexico, and the United States under the auspices of the Commission for Environmental Cooperation (CEC 2008).

These efforts have proven insufficient to reverse declines, as demonstrated through the colony loss and butterfly population metrics. To boost Federal engagement with the increased resources necessary to combat the declines, the President's Budget request to Congress for Fiscal Year (FY) 2016 includes major increases over the FY 2015 Enacted Budget for honey bee and pollinator research and habitat improvement (**Table 2**). These budget requests are in addition to agency actions to redirect, focus, and coordinate existing resources toward this challenge. A number of such actions, including development of best management practices, are being highlighted in agency implementation plans.

The FY 2016 President's Budget (**Table 2**) includes over \$82 million in funding (\$34 million above FY 2015 enacted) for DOI, EPA, and USDA, specifically targeted to address pollinator health, including Colony Collapse Disorder. Other Federal agencies also contribute to pollinator health during the conduct of some of their programs and activities. Specific agency increased resources for pollinator health include:



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**Table 2. Pollinator-specific proposed Fiscal Year (FY) 2016 budget additions relative to the Enacted FY 2015 budget for DOI, EPA, and USDA (\$ Million).**

Agency	Program	FY 2015 Enacted	FY 2016 Budget	Change from 15 Enacted to 16 Budget
DOI	U.S. Geological Survey (USGS)	0.00	1.56	1.56
	DOI Total	0.00	1.56	1.56
EPA	Office of Pesticide Programs	0.00	1.50	1.50
	State and Tribal Assistance Grants	0.00	0.50	0.50
	EPA Total	0.00	2.00	2.00
USDA	National Agricultural Statistics Service (NASS)	2.40	2.90	0.50
	Agricultural Research Service (ARS)	14.19	21.19	7.00
	National Institute of Food and Agriculture (NIFA)	9.66	31.50	21.84
	Economic Research Service (ERS)	0.28	0.28	0.00
	Land Management Programs			
	Farm Service Agency (FSA) Conservation Reserve Program (CRP)	18.00	18.06	0.06
	Natural Resource Conservation Service (NRCS) Environmental Quality Incentives Program (EQIP)	3.00	4.00	1.00
	Animal and Plant Health Inspection Service (APHIS)	1.00	1.00	0.00
	USDA Total	48.52	78.93	30.41
Agencies Total		48.53	82.49	33.96

- U.S. Department of the Interior: Includes \$1.56 million in new funding for the USGS to support research priorities identified through the 2014 Presidential Memorandum on Pollinator Health, including the development of studies, monitoring programs, and decision-support tools for land and resource management agencies, and pollinator habitat models.
- U.S. Environmental Protection Agency: Includes \$1.5 million to further the study of acute toxicity amongst honey bee populations and explore additional risk management options, and \$500,000 to augment the work of states and tribes to develop pollinator protection plans.
- U.S. Department of Agriculture: Includes \$56 million in research and associated statistical survey programs, including in-house research through ARS, agreements through APHIS, and grants (mainly through a competitive peer-reviewed process) through NIFA, with much of the funding going to land grant institutions to support local and regional pollinator issues at all levels (national, regional, and local), including organic production. Within USDA's suite of voluntary conservation programs, the budget continues to leverage funding within the Environmental Quality Incentives Program and to enhance Conservation Reserve Program covers to increase access to nutritious forage for pollinators in a targeted multi-state core area that is home to more than 65% of the Nation's managed honeybee population during the prime summer forage months (North Dakota, South Dakota, Minnesota, Wisconsin, and Michigan). It also continues

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the FY15 budget proposal to monitor existing enrollment in CRP pollinator initiatives, document and quantify the benefits to honey bees and wild pollinators, identify ways to increase the pollinator benefits from CRP land, delineate core habitat areas, and determine the appropriate mechanisms to nearly double the CRP acreage enrolled in pollinator initiatives to 200,000 acres.